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(54) Title: L-N^{6}-(1-IMINOETHYL)LYSINE DERIVATIVES USEFUL AS NITRIC OXIDE SYNTHASE INHIBITORS

(57) Abstract

There is disclosed a novel amino glycol derivatives of L-N^{6}-(1-iminoethyl)lysine, pharmaceutical compositions containing these novel compounds, and to their use in therapy, in particular their use as nitric oxide synthase inhibitors.

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L-N^6-(1-IMINOETHYL)LYSINE DERIVATIVES USEFUL AS NITRIC OXIDE SYNTHASE INHIBITORS

RELATED APPLICATION

This application is a continuation-in-part of U.S. Application Serial No. 08/209,094 filed March 10, 1994.

Background of the Invention

Field of the Invention

The present invention relates to novel amino glycol derivatives of L-N^6-(1-iminoethyl)lysine, pharmaceutical compositions containing these novel compounds, and to their use in therapy, in particular their use as nitric oxide synthase inhibitors.

Related Art

It has been known since the early 1980's that the vascular relaxation brought about by acetycholine is dependent on the presence of the endothelium and this activity was ascribed to a labile humoral factor termed endothelium-derived relaxing factor (EDRF). The activity of nitric oxide (NO) as a vasodilator has been known for well over 100 years and NO is the active component of amylnitrite, glyceryltrinitrite and other nitrovasodilators. The recent identification of EDRF as NO has coincided with the discovery of a biochemical pathway by which NO is synthesized from the amino acid L-arginine by the enzyme NO synthase.

NO is the endogenous stimulator of the soluble guanylate cyclase and is involved in a number of biological actions in addition to endothelium-dependent relaxation including cytotoxicity of phagocytic cells and cell-to-cell communication in the central nervous system (see Moncada et

al. Biochemical Pharmacology, 38, 1709-1715 (1989) and Moncada et al. Pharmacological Reviews, 43, 109-142 (1991). It is now thought that excess NO production may be involved in a number of conditions, particularly conditions which involve systemic hypotension such as toxic shock and therapy with certain cytokines.

The synthesis of NO from L-arginine can be inhibited by the L-arginine analogue, L-N-monomethyl-arginine (L-NMMA) and the therapeutic use of L-NMMA for the treatment of toxic shock and other types of systemic hypotension has been proposed (WO 91/04024 and GB-A-2240041). The therapeutic use of certain other NO synthase inhibitors apart from L-NMMA for the same purpose has also been proposed in WO 91/04024 and in EP-A-0446699.

It has recently become apparent that there are at least three types of NO synthase as follows:

(i) a constitutive, Ca^{++}/calmodulin dependent enzyme, located in the endothelium, that releases NO in response to receptor or physical stimulation.

(ii) a constitutive, Ca^{++}/calmodulin dependent enzyme, located in the brain, that releases NO in response to receptor or physical stimulation.

(iii) a Ca^{++} independent enzyme which is induced after activation of vascular smooth muscle, macrophages, endothelial cells, and a number of other cells by endotoxin and cytokines. Once expressed this inducible NO synthase synthesizes NO for long periods.

The NO released by the constitutive enzymes acts as a transduction mechanism underlying several physiological responses. The NO produced by the inducible enzyme is a cytotoxic molecule for tumor cells and invading microorganisms. It also appears that the adverse effects of

excess NO production, in particular pathological vasodilation and tissue damage, may result largely from the effects of NO synthesized by the inducible NO synthase.

There is also a growing body of evidence that NO may be involved in the degeneration of cartilage which takes place in certain conditions such as arthritis and it is also known that NO synthesis is increased in rheumatoid arthritis. Accordingly, further conditions in which there is an advantage in inhibiting NO production from L-arginine include autoimmune and/or inflammatory conditions affecting the joints, for example arthritis, inflammatory bowel disease, cardiovascular ischemia, diabetes, hyperalgesia (allodynia), cerebral ischemia (both focal ischemia, thrombotic stroke and global ischemia, secondary to cardiac arrest), and other CNS disorders mediated by NO.

Futher conditions in which there is an advantage in inhibiting NO production from L-arginine include systemic hypotension associated with septic and/or toxic shock induced by a wide variety of agents; therapy with cytokines such as TNF, IL-1 and IL-2; and as an adjuvant to short term immunosuppression in transplant therapy.

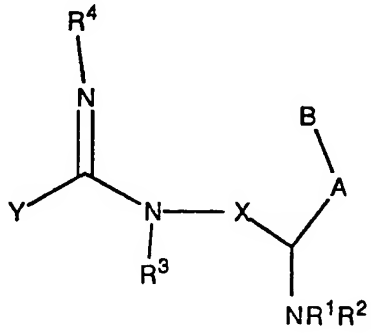
Some of the NO synthase inhibitors proposed for therapeutic use so far, and in particular L-NMMA, are non-selective in that they inhibit both the constitutive and the inducible NO synthase. Use of such a non-selective NO synthase inhibitor requires that great care be taken in order to avoid the potentially serious consequences of over-inhibition of the constitutive NO-synthase including hypertension and possible thrombosis and tissue damage. In particular, in the case of the therapeutic use of L-NMMA for the treatment of toxic shock it has been recommended that the patient must be subject to continuous blood pressure

monitoring throughout the treatment. Thus, while non-selective NO synthase inhibitors have therapeutic utility provided that appropriate precautions are taken, NO synthase inhibitors which are selective in the sense that they inhibit the inducible NO synthase to a considerably greater extent than the constitutive isoforms of NO synthase would be of even greater therapeutic benefit and easier to use.

WO94/12165, WO94/14780, WO93/13055, EP0446699A1 and U.S. Patent No. 5,132,453 disclose compounds that inhibit nitric oxide synthesis and preferentially inhibit the inducible isoform of nitric oxide synthase. The disclosures of which are hereby incorporated by reference in their entirety as if written herein.

Summary of the Invention

In accordance with the present invention novel amino glycol derivatives of L-N^6-(1-iminoethyl)lysine derivatives are provided. These novel inhibitor compounds can be represented by the following chemical formula. A compound or a pharmaceutically acceptable salt, prodrug or ester therof having the formula:



Y is a hydrogen, lower alkyl radical, lower alkenyl radical,lower alkynyl radical, aromatic hydrocarbon radical, alicyclic hydrocarbon radical, amino, heterocyclyl radical

in which 1 to about 4 heteroatoms are independently selected from oxygen, nitrogen and sulfur, wherein all said radicals may optionally be substituted with hydrogen, cyano, lower alkyl, nitro, amino, alicyclic hydrocarbon radicals, or aromatic hydrocarbon radicals which may be optionally substituted with lower alkyl;

X is lower alkyl radical, lower alkenyl radical, lower alkynyl radical, aromatic hydrocarbon radical, $(CH_2)_mQ(CH_2)_n$, where m= 1-3, n = 1-3, and Q is sulfur, sulfinyl, sulfonyl or oxygen, C=O, lower alkynyl radical, aromatic hydrocarbon radical, alicyclic hydrocarbon radical or heterocyclyl radicals in which 1 to about 4 heteroatoms are independently selected from oxygen, nitrogen and sulfur, wherein all said radicals are optionally substituted with hydrogen, halogen and lower alkyl;

R^1, R^2, R^3 and R^4 are independently selected from the group consisting of hydrogen and lower alkyl;

A is a lower alkyl radical, lower alkenyl radical,lower alkynyl radical, alicyclic hydrocarbon radical, C=O, aromatic hydrocarbon radical or heterocyclyl radical in which 1 to about 4 heteroatoms are independently selected from oxygen, nitrogen and sulfur, wherein all said radicals are optionally substituted with hydrogen, lower alkyl, hydroxyl, lower alkoxy, alkoxycarbonyl, alkylaryloxy, thiol, lower thioalkoxy, thioalkylaryloxy, thioaryloxy, sulfinylalkyl, sulfinylalkylaryl, sulfinylaryl, sulfonylalkyl, sulfonylalkylaryl, sulfonylaryl, halogen, aromatic hydrocarbon radicals, or alicyclic hydrocarbon radicals;

B can be hydrogen, lower alkyl radical, lower alkenyl radical, lower alkynyl radical, lower alkoxy radical,

hydroxy, alkoxycarbonyl, alkylaryloxy, thiol, lower thioalkoxy, lower thioalkylaryloxy, thioaryloxy, sulfinylalkyl, sulfinylalkylaryl, sulfinylaryl, sulfonylalkyl, sulfonylalkylaryl, sulfonylaryl, aromatic hydrocarbon radical, alicyclic hydrocarbon radical, or heterocyclyl radical in which 1 to about 4 heteroatoms are independently selected from oxygen, nitrogen and sulfur wherein all said radicals are optionally substituted with hydrogen, lower alkyl, hydroxyl, lower alkoxy, halogen, aromatic hydrocarbon radicals,or alicyclic hydrocarbon radical, or

B can be $C(=O)OR^5$, $C(=O)NR^5R^6$, $P(=O)(OR^5)(OR^6)$, NHOH, $N(OH)C(=O)NR^5R^6$, $NR^5C(=O)NR^6R^7$, $NR^5C(=O)N(OH)R^6$, $C(=O)NHOH$,

where

R^5 is hydrogen, lower alkyl radical, aromatic hydrocarbon radical, or alicyclic hydrocarbon radical wherein all said radicals are optional substituted with lower alkyl, lower alkenyl;

R^6 is hydrogen, lower alkyl radical, aromatic hydrocarbon radical, or alicyclic hydrocarbon radical wherein all said radicals are optional substituted with lower alkyl, lower alkenyl; and

R^7 is hydrogen, lower alkyl radical, aromatic hydrocarbon radical, or alicyclic hydrocarbon radical wherein all said radicals are optional substituted with lower alkyl, lower alkenyl;

with the proviso that when A is C=O, B may not be hydroxy or alkoxy.

(I)

A is preferably lower alkyl which is substituted as indicated above.

In another broad aspect, the present invention is directed to inhibiting nitric oxide synthesis in a subject in need of such inhibition or treatment by administering a compound of Formula (I) which preferentially inhibits the inducible isoform of nitric oxide synthase over the constitutive isoform of nitric oxide synthase, in a nitric oxide synthesis inhibiting amount to such subject.

The invention further relates to a pharmaceutical composition comprising a compound from Formula (I).

Compounds and compositions defined above have usefulness as inhibitors of nitric oxide synthase. These compounds also preferentially inhibit the inducible form over the constitutive form by at least 3 fold.

Conditions in which there is an advantage in inhibiting NO production from L-arginine include systemic hypotension associated with septic and/or toxic shock induced by a wide variety of agents; therapy with cytokines such as TNF, IL-1 and IL-2; and as an adjuvant to short term immunosuppression in transplant therapy. Further conditions in which there is an advantage in inhibiting NO production from L-arginine include autoimmune diseases and/or inflammatory conditions such as those affecting the joints, for example arthritis or inflammatory bowel disease, cardiovascular ischemia, diabetes, cerebral ischemia and other CNS disorders mediated by NO.

A preferred embodiment of the present invention is a compound of the formula (I) wherein

Y is hydrogen or lower alkylene

X is lower alkylene from 3-5 carbon

R^1, R^2, R^3, and R^4 are independently selected from the group consisting of hydrogen or lower alkyl

A is lower alkylene from 2-4 carbons sustituted with hydroxyl

B is hydroxyl.

It is preferred that Y is methyl, X is preferably butylene, R^1, R^2, R^3, and R^4 are preferably hydrogen, A is preferably ethylene or isopropylene substituted with hydroxyl and B is preferably hydroxyl (OH).

The present invention includes compounds of formula (I) in the form of salts, in particular acid addition salts. Suitable salts include those formed with both organic and inorganic acids. Such acid addition salts will normally be pharmaceutically acceptable although salts of non-pharmaceutically acceptable salts may be of utility in the preparation and purification of the compound in question. Thus, preferred salts include those formed from hydrochloric, hydrobromic, sulfuric, citric, tartaric, phosphoric, lactic, acetic, succinic, fumaric, maleic, methanesulfonic, ethanesulfonic,p-toluenesulfonic, benzenesulfonic and the like. (See, for example, S. M. Berge et al., Pharmaceutical Salts, *J. Pharm. Sci.*, **1977**, *66*, 1-19.) Salts of the compounds of formula (I) can be made by reacting the appropriate compound in the form of the free base with the appropriate acid.

While it may be possible for the compounds of formula (I) to be administered as the raw chemical, it is preferable to present them as a pharmaceutical formulation. According to a further aspect, the present invention provides a pharmaceutical formulation comprising a compound of formula (I) or a pharmaceutically acceptable salt or solvate

thereof, together with one or more pharmaceutically acceptable carriers thereof and optionally one or more other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The formulations include those suitable for oral, parenteral (including subcutaneous, intradermal, intramuscular, intravenous and intraarticular), rectal and topical (including dermal, buccal, sublingual and intraocular) administration although the most suitable route may depend upon for example the condition and disorder of the recipient. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association a compound of formula (I) or a pharmaceutically acceptable salt or solvate thereof ("active ingredient") with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both and then, if necessary, shaping the product into the desired formulation.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein.

Formulations for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain antioxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline, water-for-injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Formulations for rectal administration may be presented as a suppository with the usual carriers such as cocoa butter or polyethylene glycol.

Formulations for topical administration in the mouth, for example buccally or sublingually, include lozenges

comprising the active ingredient in a flavored basis such as sucrose and acacia or tragacanth, and pastilles comprising the active ingredient in a basis such as gelatin and glycerin or sucrose and acacia.

Preferred unit dosage formulations are those containing an effective dose, as hereinbelow recited, or an appropriate fraction thereof, of the active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above, the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavoring agents.

The compounds of the invention may be administered orally or via injection at a dose of from 0.001 to 2500 mg/kg per day. The dose range for adult humans is generally from 0.005 mg to 10 g/day. Tablets or other forms of presentation provided in discrete units may conveniently contain an amount of compound of the invention which is effective at such dosage or as a multiple of the same, for instance, units containing 5 mg to 500 mg, usually around 10 mg to 200 mg.

The compounds of formula (I) are preferably administered orally or by injection (intravenous or subcutaneous). The precise amount of compound administered to a patient will be the responsibility of the attendant physician. However, the dose employed will depend on a number of factors, including the age and sex of the patient, the precise disorder being treated, and its severity. Also, the route of administration may vary depending on the condition and its severity.

As utilized herein, the term "lower alkyl", alone or in combination, means an acyclic alkyl radical containing from 1 to about 10, preferably from 1 to about 8 carbon atoms and more preferably 1 to about 6 carbon atoms. Examples of such radicals include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, iso-amyl, hexyl, octyl and the like.

The term "lower alkenyl" refers to an unsaturated acyclic hydrocarbon radical in so much as it contains at least one double bond. Such radicals containing from about 2 to about 10 carbon atoms, preferably from about 2 to about 8 carbon atoms and more preferably 2 to about 6 carbon atoms. Examples of suitable alkenyl radicals include propylenyl, buten-1-yl, isobutenyl, pentenylen-1-yl, 2-2-methylbuten-1-yl, 3-methylbuten-1-yl, hexen-1-yl, hepten-1-yl, and octen-1-yl, and the like.

The term "lower alkynyl" refers to an unsaturated acyclic hydrocarbon radical in so much as it contains one or more triple bonds, such radicals containing about 2 to about 10 carbon atoms, preferably having from about 2 to about 8 carbon atoms and more preferably having 2 to about 6 carbon atoms. Examples of suitable alkynyl radicals include ethynyl, propynyl, butyn-1-yl, butyn-2-yl, pentyn-1-yl, pentyn-2-yl, 3-methylbutyn-1-yl, hexyn-1-yl, hexyn-2-yl, hexyn-3-yl, 3,3-dimethylbutyn-1-yl radicals and the like.

The term "alicyclic hydrocarbon" or "cycloalkyl" means a aliphatic radical in a ring with 3 to about 10 carbon atoms, and preferably from 3 to about 6 carbon atoms. Examples of suitable alicyclic radicals include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclohexenyl and the like.

The term "aromatic hydrocarbon radical" means 4 to about 16 carbon atoms,preferably 6 to about 12 carbon atoms,

more preferably 6 to about 10 carbon atoms. Examples of suitable aromatic hydrocarbon radicals include phenyl,naphthyl, and the like.

The term "aryl" as used herein means 5- and 6-membered single-aromatic radicals which may include from zero to four heteroatoms. Representative aryls include phenyl, thienyl, furanyl, pyridinyl, (is)oxazoyl and the like.

The term DCM means dichloromethane.

The term DEAD means diethyl azodicarboxylate.

The term DIBAL-H means diisobutylaluminum hydride.

The term DMAP means dimethylaminopyridine.

The term DMSO means dimethylsulfoxide.

The term EDC means 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride.

The term "heterocyclyl radical" means a saturated or unsaturated cyclic hydrocarbon radical including aromatic systems with 4 to about 10 carbon atoms, preferably about 5 to about 6; wherein 1 to about 4 carbon atoms are replaced by nitrogen, oxygen or sulfur. The "heterocyclic radical" may be fused to an aromatic hydrocarbon radical. Suitable examples include pyrrolyl, pyridinyl, pyrazolyl, triazolyl, pyrimidinyl, pyridazinyl, oxazolyl, isoxazolyl, thiazolyl, imidazolyl, indolyl, thienyl, furanyl, tetrazolyl, 2-pyrrolinyl, 3-pyrrolinyl, pyrrolindinyl, 1,3-dioxolanyl, 2-imidazonlinyl, imidazolidinyl, 2-pyrazolinyl, pyrazolidinyl, isoxazolyl, isothiazolyl, oxadiazolyl, triazolyl, thiadiazolyl, 2H-pyranyl, 4H-pyranyl, piperidinyl, 1,4-dioxanyl, morpholinyl, 1,4-dithianyl, thiomorpholinyl, pyrazinyl, piperazinyl, triazinyl, 1,3,5-trithianyl, benzo(b)thiophenyl, benzimidazolyl, quinolinyl, and the like.

The term HOBT means N-hydroxybenzotriazole.

The term "lower alkoxy", alone or in combination, means an alkyl ether radical wherein the term alkyl is as defined above and most preferably containing 1 to about 4 carbon

atoms. Examples of suitable alkyl ether radicals include methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, iso-butoxy, sec-butoxy, tert-butoxy and the like.

The term "lower thioalkoxy", alone or in combination, means an alkyl thioether radical wherein the term alkyl is as defined above and most preferably containing 1 to about 4 carbon atoms. Examples of suitable alkyl thioether radicals include thiomethoxy, thioethoxy, thio-n-propoxy, thio-i-propoxy, thio-n-butoxy, thio-iso-butoxy, thio-sec-butoxy, thio-tert-butoxy and the like.

The term alkoxycarbonyl as used herein means an alkoxy group, as defined above, having a carbonyl (C=O) group attached.

The term "halogen" means fluorine, chlorine, bromine or iodine.

The term mcpba means m-chloroperbenzoic acid.

The term NMM means N-methylmorpholine.

The term NMMO means 4-methylmorpholine N-oxide.

The term "prodrug" refers to a compound that is made more active *in vivo*.

The term sulfinyl means SO.

The term sulfonyl means SO_2.

The term TEA means triethylamine.

The term $TMSN_3$ means azidotrimethylsilane.

As used herein, reference to "treatment" of a patient is intended to include prophylaxis.

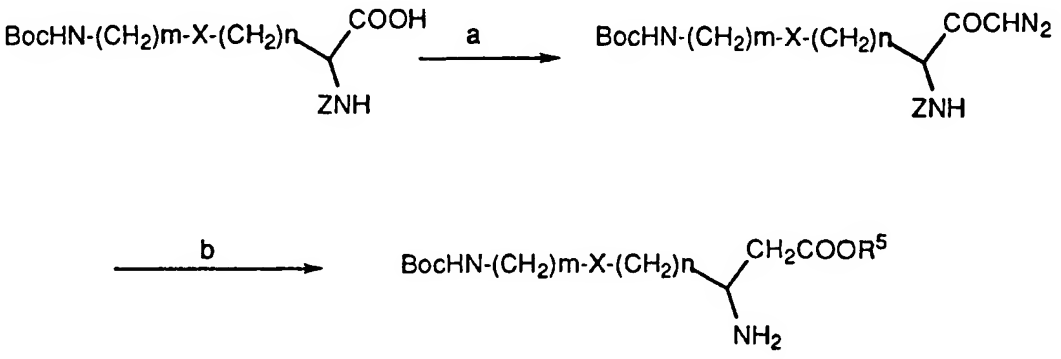
All references, patents or applications, U.S. or foreign, cited in the application are hereby incorporated by reference as if written herein.

Compounds of the present invention can exist in geometric or stereoisomeric forms. The present invention contemplates all such compounds, including cis- and trans-geometric isomers, E- and Z-geometric isomers, R- and S-enantiomers, diastereomers, d-isomers, l-isomers, the

racemic mixtures thereof and other mixtures thereof, as falling within the scope of the invention.

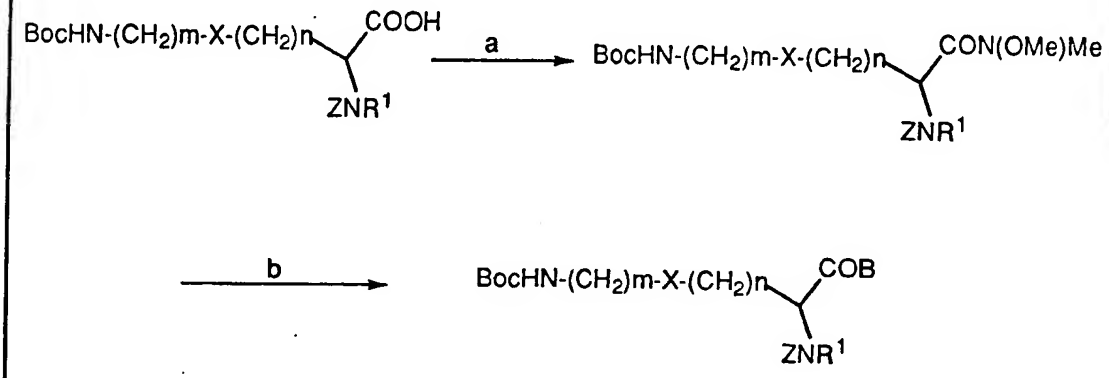
Disclosed are twenty eight general synthetic processes useful in the preparation of the compounds of the present invention.

Scheme 1



(a) (i) i-butyl chloroformate, NMM, THF; (ii) CH_2N_2; (b) Ag benzoate, R^5OH, TEA.

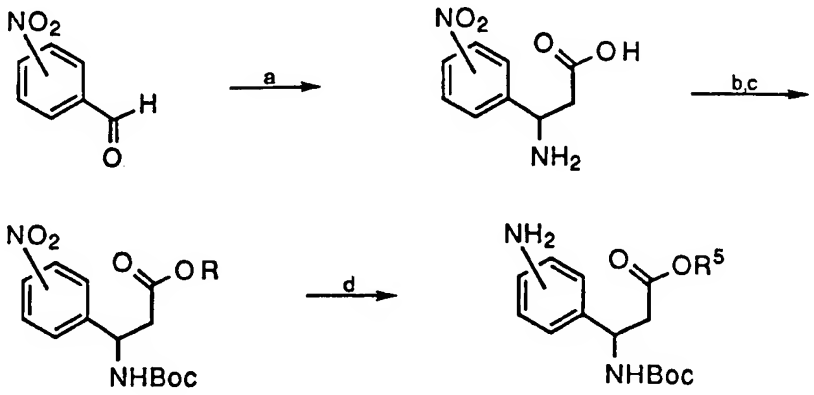
Scheme 2



(a) N,O-Dimethylhydroxylamine HCl, EDC, HOBT, TEA, DMF; (b) B*Li, THF.

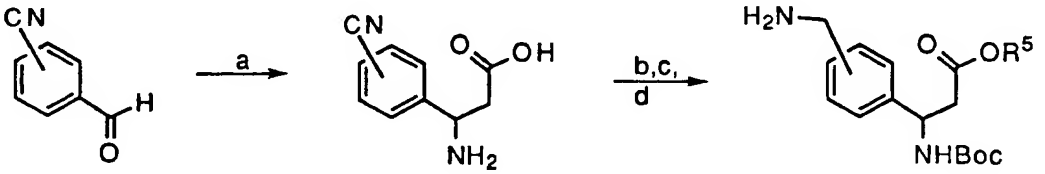
* see generic claims for B = alkyl, aryl, heteroaryl

Scheme 3



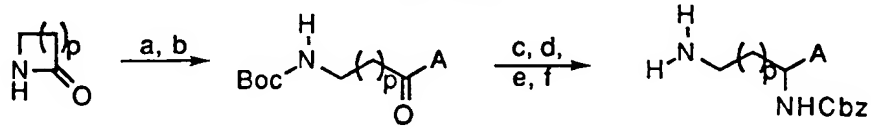
(a) ammonium acetate, malonic acid, acetic acid; (b) di-t-butyl dicarbonate, NaOH/dioxane; (c) $NaHCO_3$, DMF, R^5I; (d) H_2 Pd/C.

Scheme 4



(a) ammonium acetate, malonic acid, acetic acid; (b) di-t-butyl dicarbonate, NaOH/dioxane; (c) $NaHCO_3$, DMF, R^5I; (d) H_2 Pd/C.

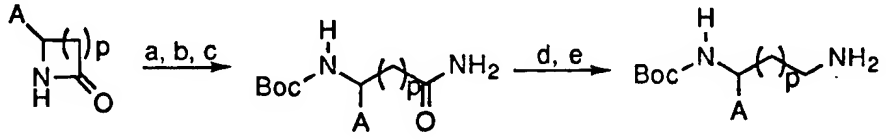
Scheme 5



(a) di-t-butyl dicarbonate, DMAP, THF; (b) A*Li; (c) hydroxylamine hydrochloride (d) H_2 Pd/C e) CbzCl (f) HCl/dioxane.

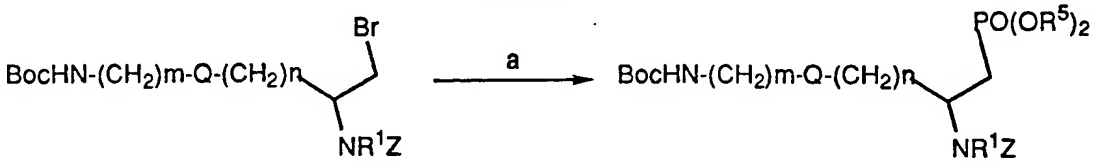
*see generic claims for A = alkyl, aryl, heteroaryl, alkaryl, alkheteroaryl.

Scheme 6



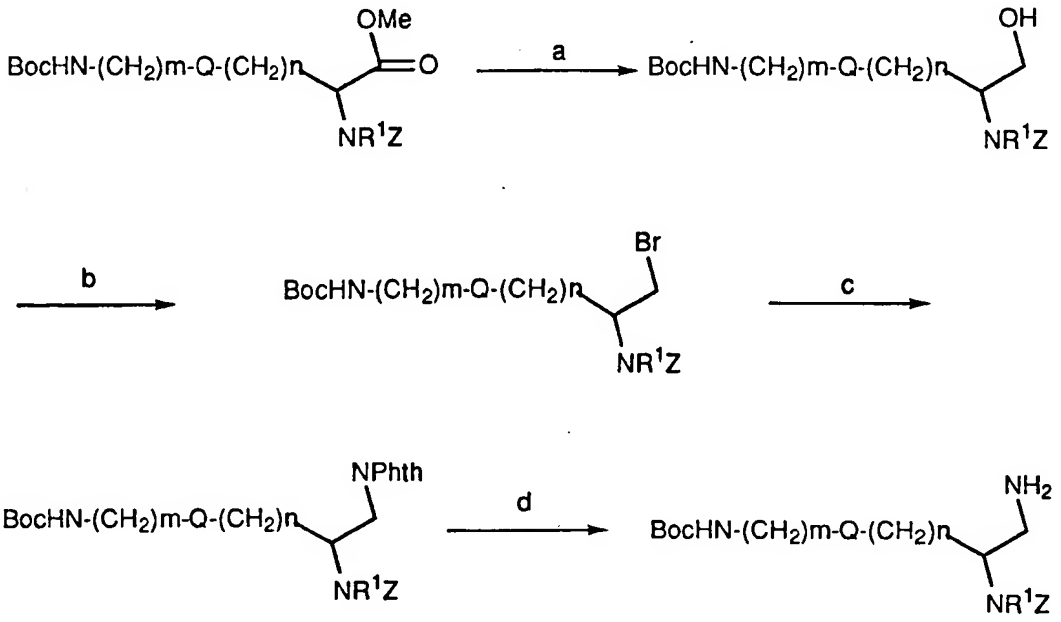
(a) di-t-butyl dicarbonate, DMAP, THF; (b) LiOH; (c) i-butyl chloroformate, ammonia (d) trifluoroacetic anhydride, Et_3N (e) H_2/Pd.

Scheme 7



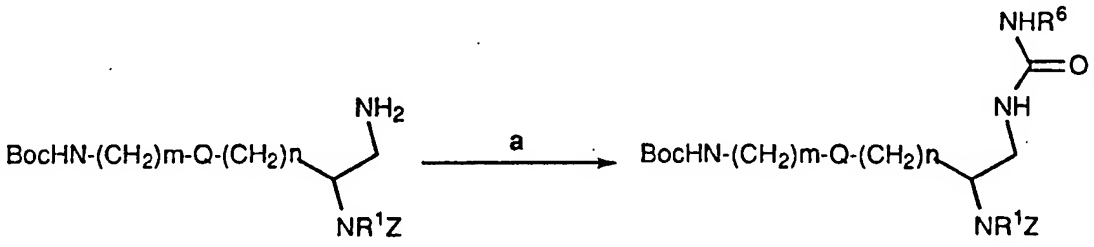
(a) $P(OR^5)_3$.

Scheme 8



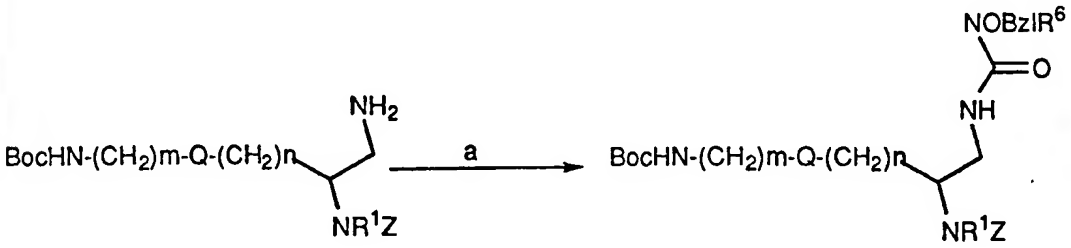
(a) DIBAL-H, toluene, -70°C, 1 h; (b) Ph_3PBr_2, DMAc, 16 h; (c) K^+NPhth, THF; (d) NH_2NH_2, EtOH.

Scheme 9



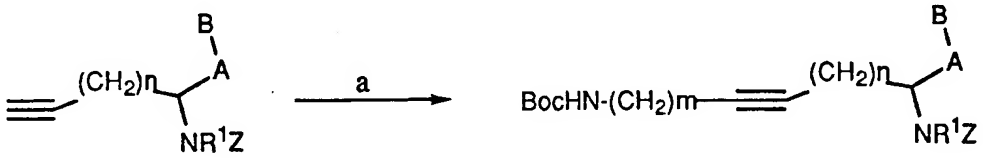
(a) O=C=NR^6, DCM.

Scheme 10



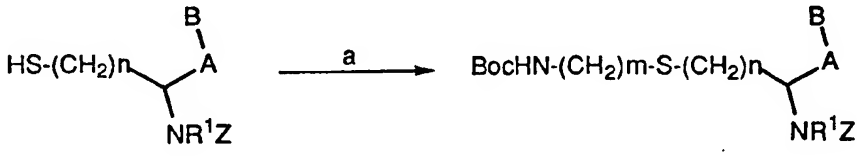
(a) carbonyldiimidiazole, $NHOBzlR^6$, DCM.

Scheme 11



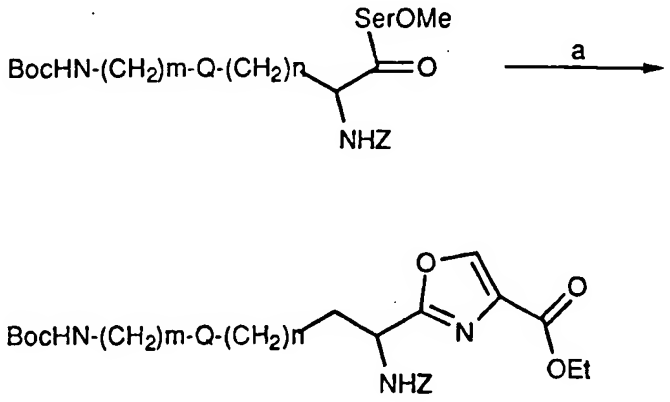
(a) n-BuLi, THF, $BocNH(CH_2)mBr$.

Scheme 12



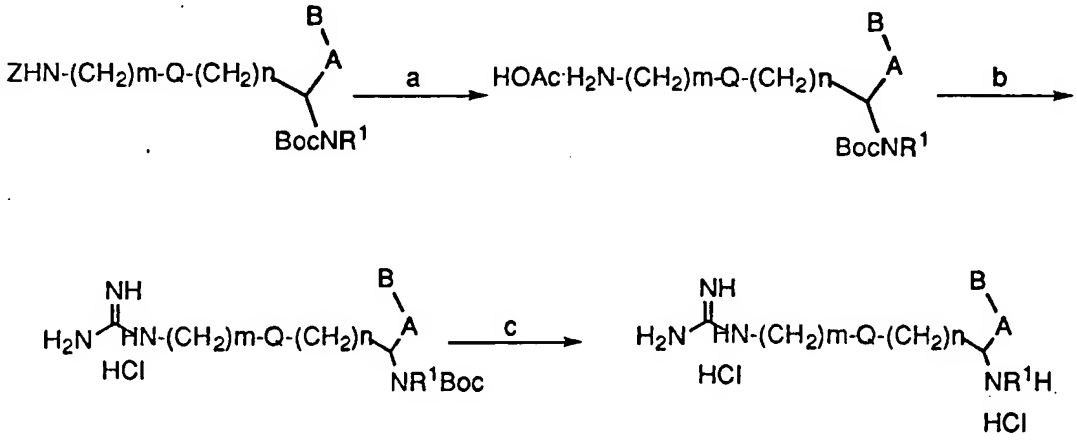
(a) NaOMe, THF, $BocNH(CH_2)mBr$.

Scheme 13



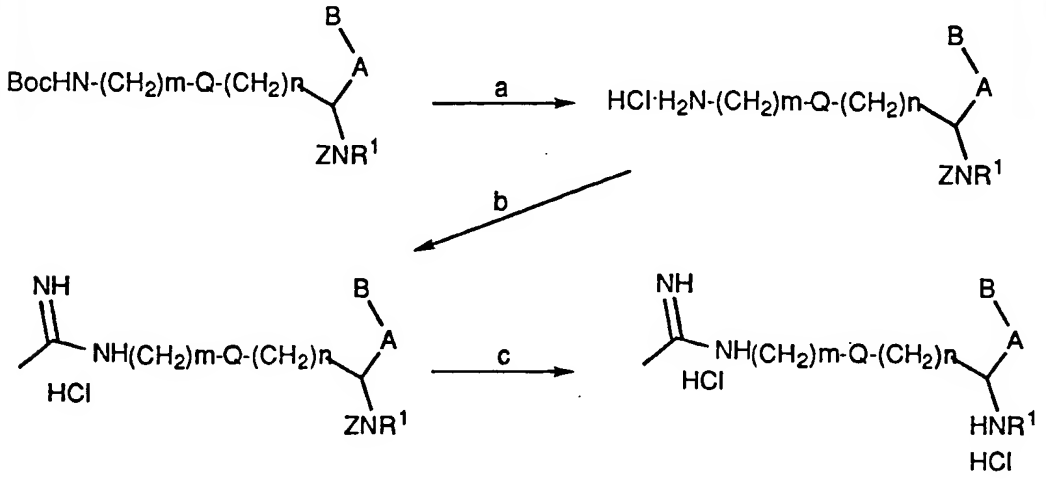
(a) (i) Ph_3P, CBr_4, (ii) $CuBr_2$.

Scheme 14



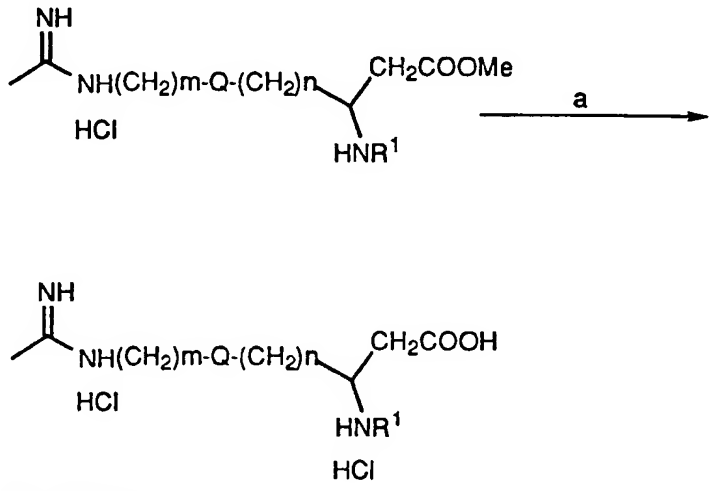
(a) H_2, Pd black, EtOH/HOAc; (b) (i) 3,5-dimethylpyrazole-1-carboxamidine nitrate, NaOH; (ii) 1N HCl; (c) HCl/dioxane, HOAc.

Scheme 15



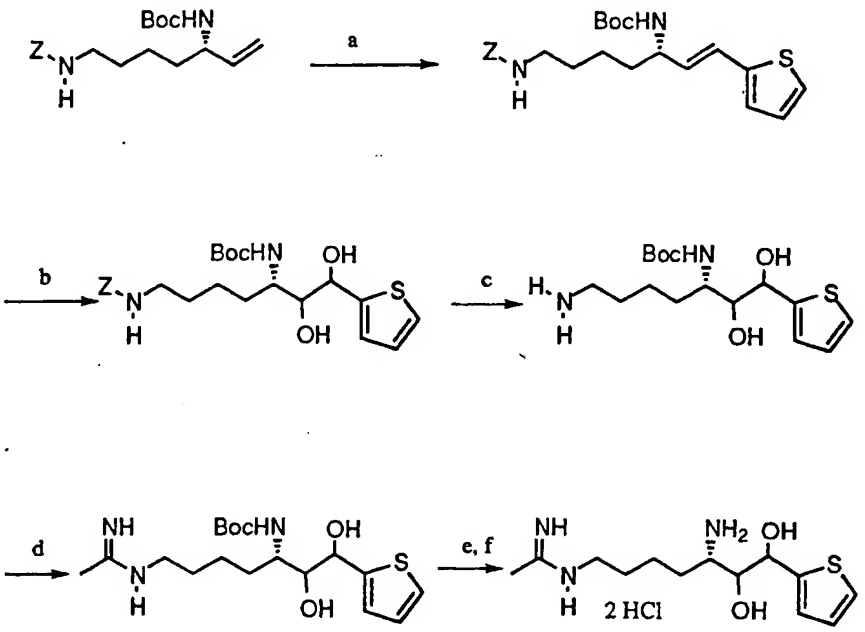
(a) HCl/dioxane, HOAc; (b) (i) methyl acetimidate, NaOH; (ii) 1 N HCl;
(c) H_2, Pd black, EtOH, HOAc.

Scheme 16



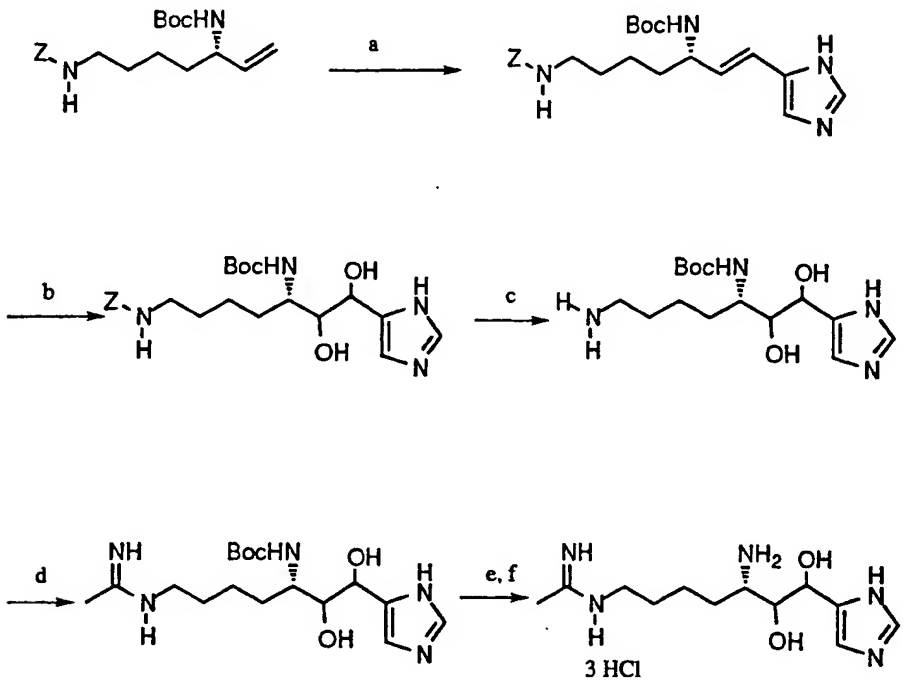
(a) 2 N HCl, Δ.

Scheme 17



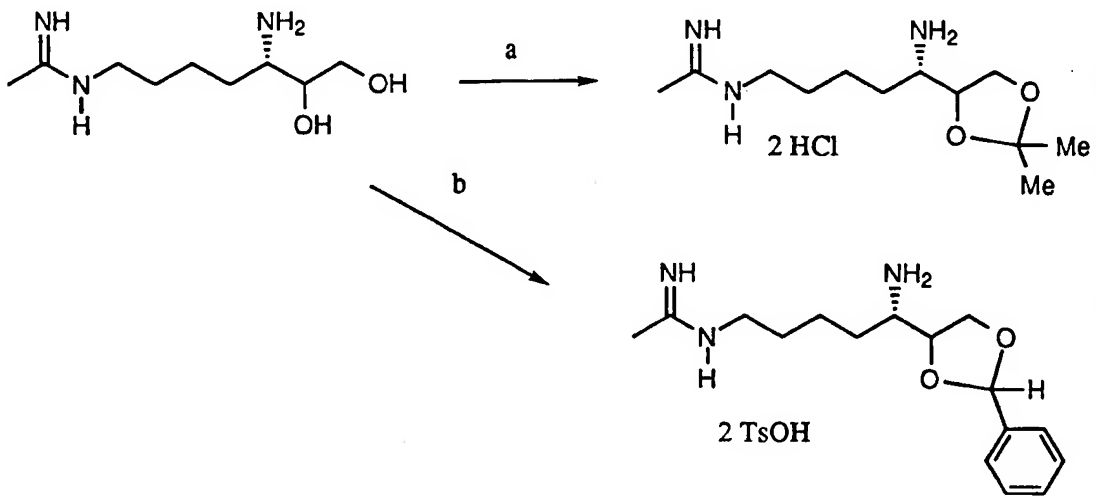
(a) $Pd(OAc)_2$/tri-o-tolylphosphine/2-bromothiophene/triethylamine; (b) OsO_4, NMMO, acetone-H_2O; (c) H_2/Pd/AcOH; (d) ethyl acetimidate HCl/EtOH; (e) HCl/Dioxane/AcOH; (f) HCl/H_2O.

Scheme 18



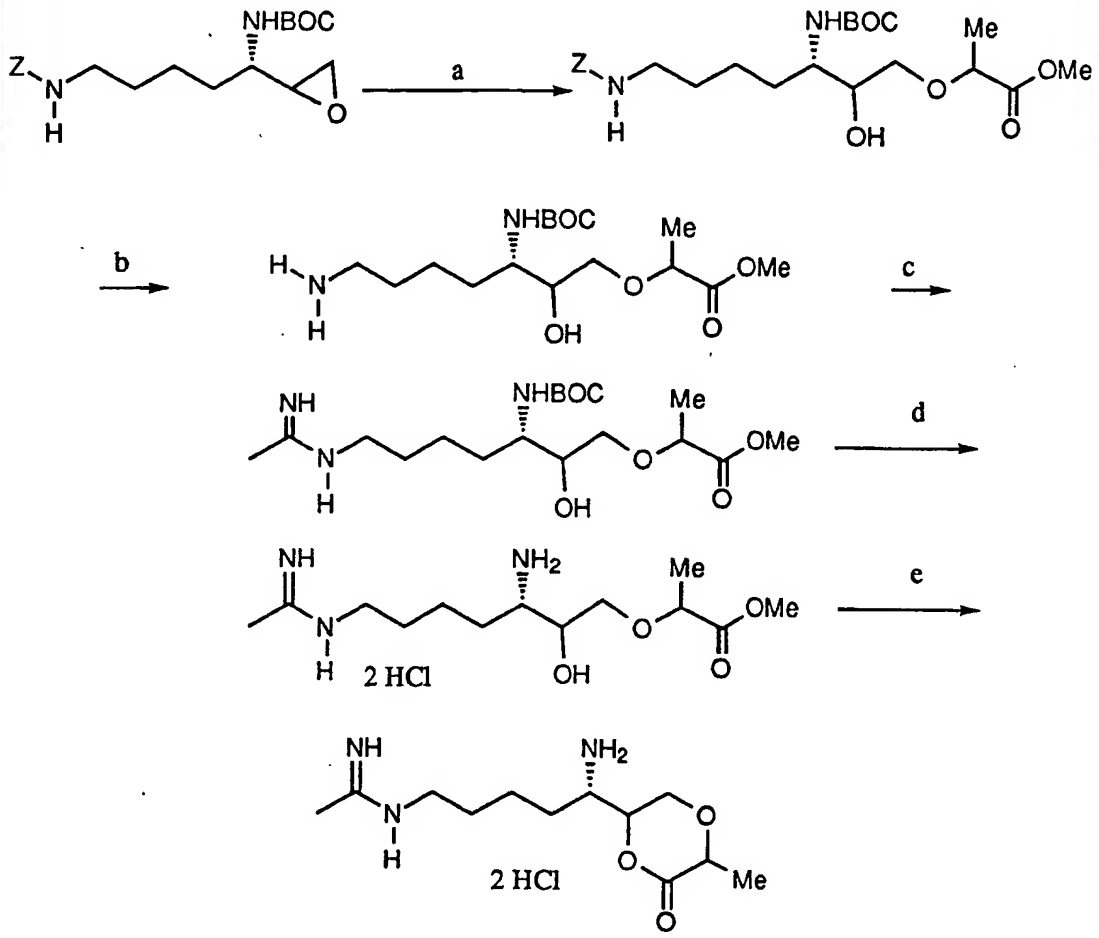
(a) $Pd(OAc)_2$/tri-o-tolylphosphine/4-bromoimidazole/triethylamine; (b) OsO_4, NMMO; (c) H_2/Pd/AcOH; (d) ethyl acetimidate HCl/EtOH; (e) HCl/Dioxane/AcOH; (f) HCl/H_2O.

Scheme 19



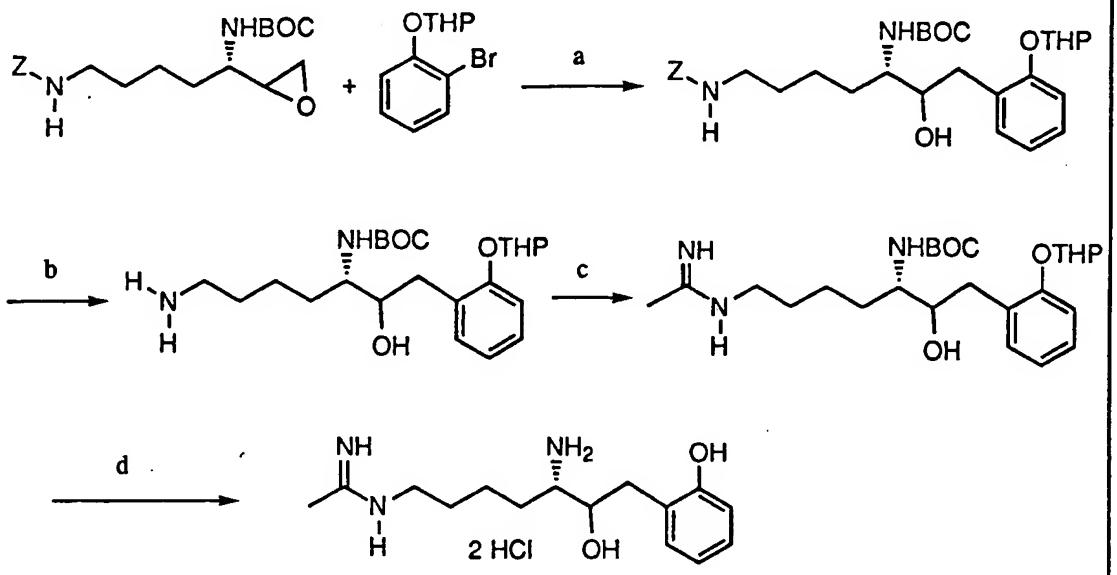
(a) 2,2-dimethoxypropane/DMF/HCl/Dioxane; (b) 1,1,1-trichloroethane/TsOH/benzaldehyde.

Scheme 20



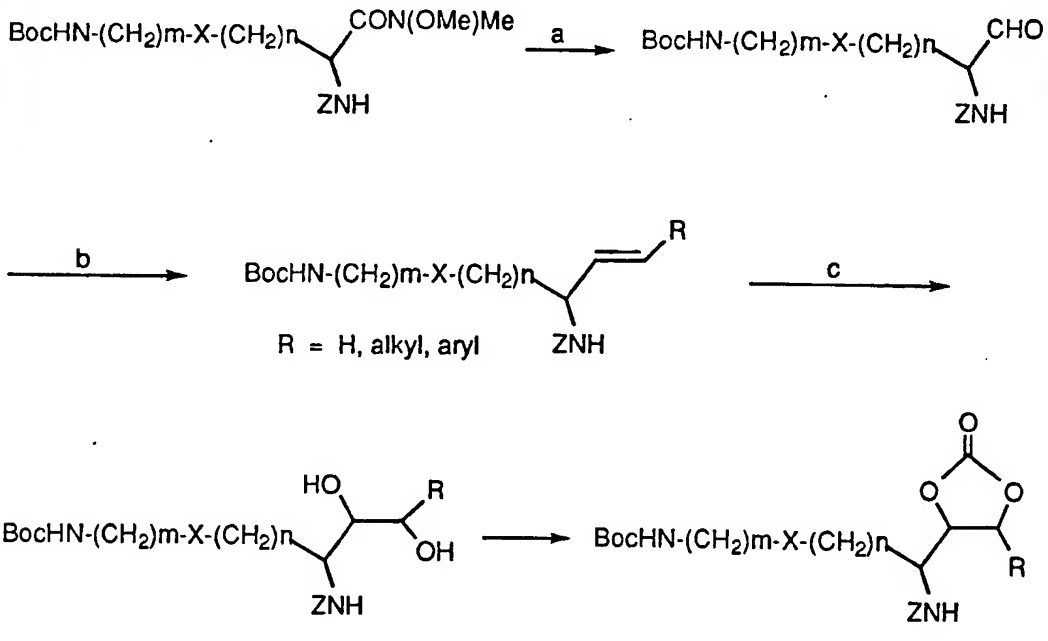
(a) NaH/DMF/$ZnCl_2$/THF/methyl lactate; (b) H_2/Pd/AcOH; (c) ethyl acetimidate HCl/EtOH; (d) HCl/Dioxane/AcOH; (e) HCl/H_2O.

Scheme 21



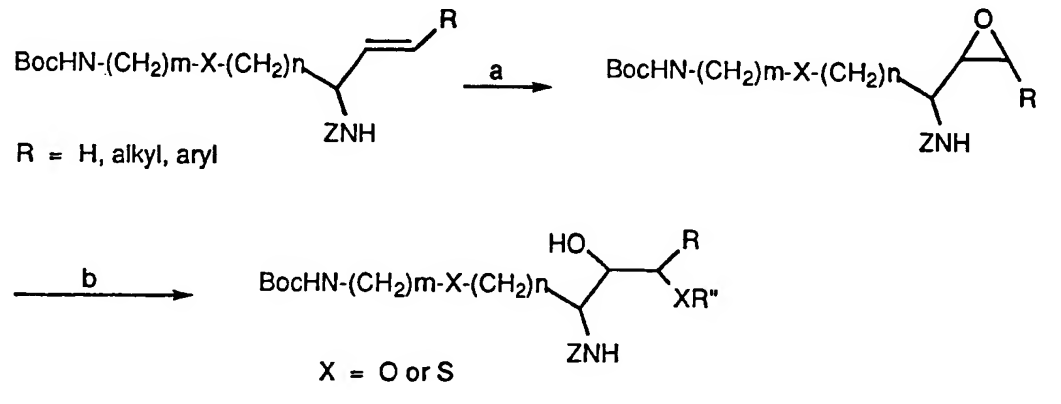
(a) n-BuLi/THF/-78 °C; (b) H_2/Pd/AcOH; (c) ethyl acetimidate HCl/EtOH; (d) HCl/Dioxane/AcOH.

Scheme 22



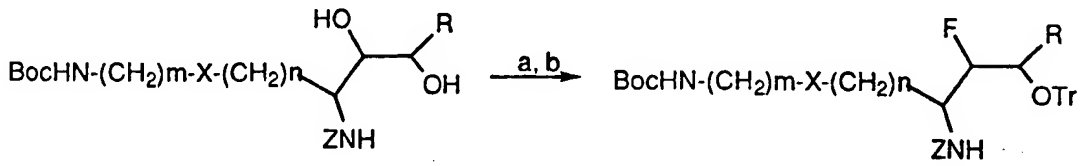
(a) $LiAlH_4$, THF, 0 °C - 20 °C; (b) $RCH=PPh_3$, THF; (c) OsO_4, NMMO, acetone-H_2O; (d) $Cl_2C=O$, pyridine.

Scheme 23



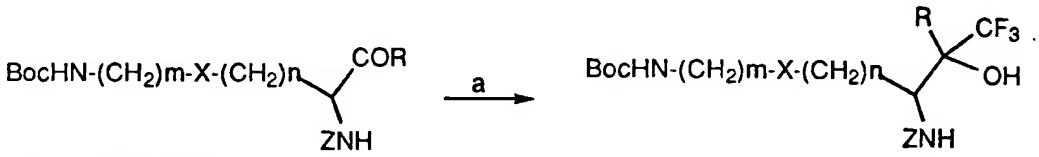
(a) mcpba, DCM, 20 °C; (b) NaSR" or NaOR", toluene-THF, Lewis acid.

Scheme 24



(a)TrCl, pyridine 20 °C; (b) Et_2NSF_3, dioxane.

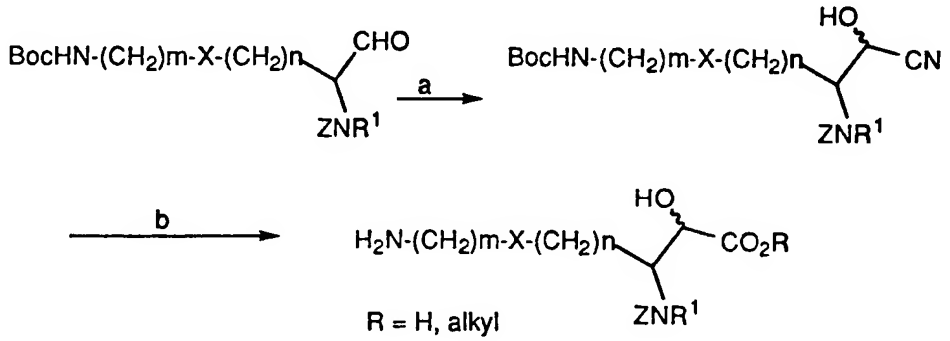
Scheme 25



R = H, alkyl, aryl

(a) CF_3I, Zn, DMF, -20 °C.

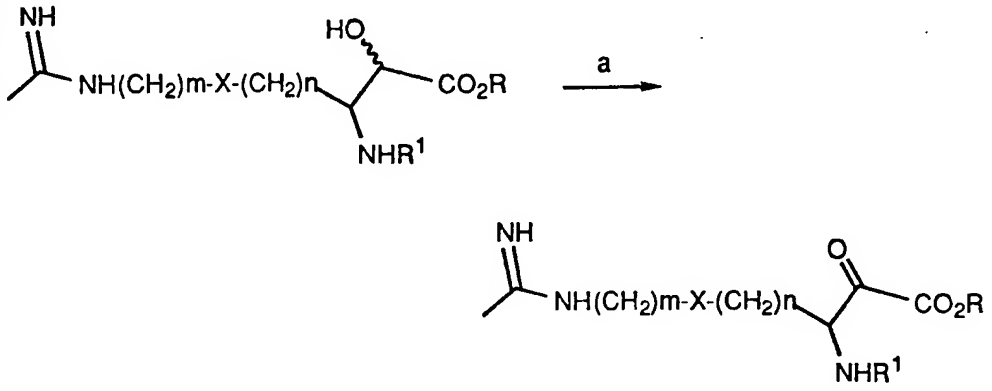
Scheme 26



R = H, alkyl

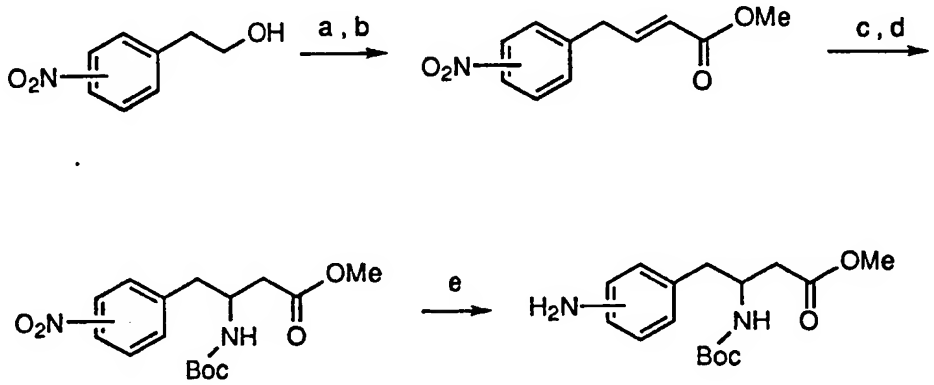
(a)KCN, $NaHSO_4$; (b) ROH, HCl or H_2O, H^+.

Scheme 27



(a) MnO_2, H_2O.

Scheme 28



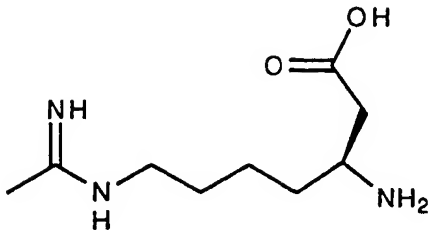
(a) $(COCl)_2$, TEA, DMSO, DCM; (b) (carbomethoxymethyl)triphenylphosphonium bromide; (c) ammonium chloride; (d) di-t-butyl dicarbonate; (e) H_2, Pd/C.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. Therefore the following preferred specific embodiments are to be construed as merely illustrative and not limitative of the remainder of the disclosure in any way whatsoever.

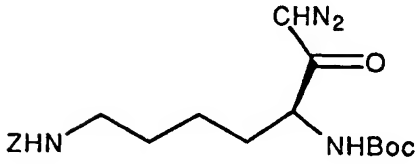
All experiments were performed under either dry nitrogen or argon. All solvents and reagents were used without further purification unless otherwise noted. The routine work-up of the reactions involved the addition of the reaction mixture to a mixture of either neutral, or acidic, or basic aqueous solutions and organic solvent. The aqueous layer was extracted n times (x) with the indicated organic solvent. The combined organic extracts were washed n times (x) with the indicated aqueous solutions, dried over anhydrous Na_2SO_4, filtered, concentrated *in vacuo*, and purified as indicated. Separations by column chromatography were achieved with conditions described by Still. (Still, W. C.; Kahn, M.; Mitra, A. Rapid Chromatograhic Technique for Preparative Separation with Moderate Resolution. *J. Org. Chem.*, **1978**, *43*, 2923-2925.) The hydrochloride salts were made from 1N HCl, HCl in ethanol (EtOH), 2 N in MeOH, or 6 N HCl in dioxane. Thin layer chromatograms were run on 0.25 mm EM precoated plates of silica gel 60 F254. High performance liquid chromatograms (HPLC) were obtained from C-8 or C-18 reverse phase columns which were obtained from several vendors. Analytical samples were dried in an Abderhalden apparatus at either 56°C or 78°C. ^1H NMR spectra were obtained from either General Electric QE-300 or Varian VXR 400 MHz spectrometer with tetramethylsilane as an internal standard. ^{13}C NMR were obtained from a Varian spectrometer at 125.8 MHz with tetramethylsilane as an internal standard.

Example 1

3S-amino-7-[(1-iminoethyl)amino]heptanoic acid

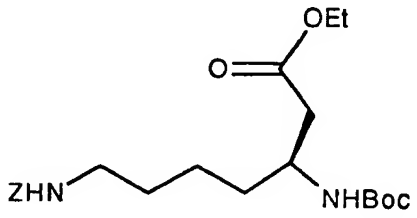


1



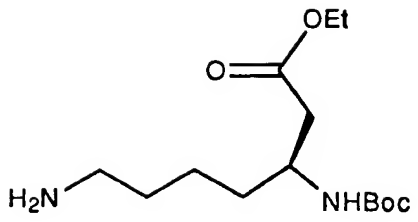
1a

1a. Boc-L-Lys(Z)-OH in 50 mL THF (3.8 g, 10 mmol) was reacted with isobutyl chloroformate (1.4 mL, (10 mmol) in the presence of NMM (1.1 mL, 10 mmol). The salt was filtered and the mixed anhydride was reacted with 25 mmol diazomethane in 100 mL Et_2O for 12 h. Solvent was evaporated to give an oil. This structure and subsequent structures were characterized by ^1H NMR.



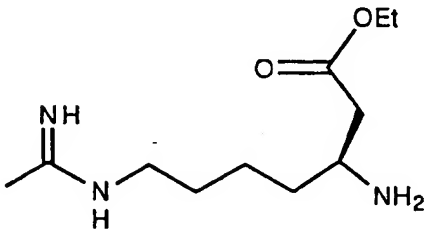
1b

1b. **1a** dissolved in 50 mL EtOH was treated with Ag benzoate (0.5 g) in the presence of TEA (5 mL) for 2 h. After filtration, the β-amino acid ester was purified by column chromatography to give 0.84 g of product.



1c

1c. **1b** (0.84 g 12 mmol) dissolved in 30 mL MeOH was reduced in the presence of 1 g ammonium formate and 0.2 g Pd black for 60 min. After filtration and evaporation, product was recovered.



1d

Ethyl 3S-amino-7-[(1-iminoethyl)amino]heptanoate

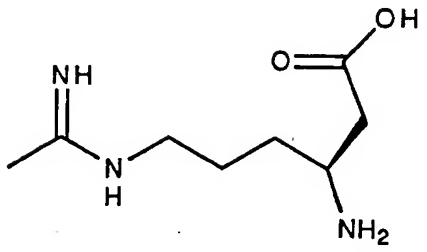
1d. **1c** in 10 mL DMF was treated with methyl acetimidate (0.692 g, 6 mmol) and N,N-diisopropylethylamine (1.05 mL, 6 mmol) overnight. Solvent was removed *in vacuo* and the residue treated with TFA (10 mL) for 30 min. The reaction was diluted with H_2O and purified by HPLC to yield 0.16 g (35.1%) of an oil. FAB MS: MH^+=230.2

1

1. **1d** (0.12 g, 0.52 mmol) dissolved in 20 mL 2N HCl was refluxed for 60 min. The reaction was diluted with H_2O and lyophilized to yield 0.107 g (100%) of an oil. FAB MS: MH^+=202.3

Example 2

3S-amino-6-[(1-iminoethyl)amino]hexanoic acid



2

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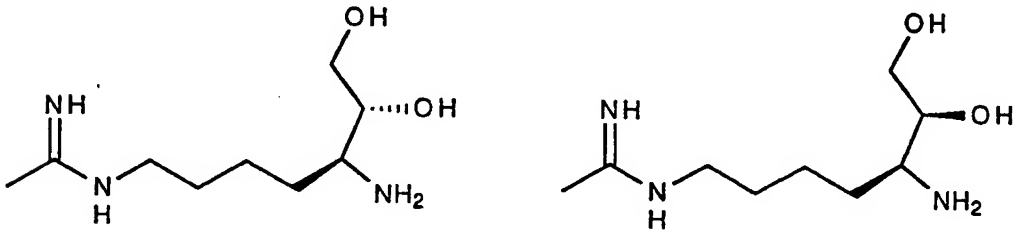
2. Example 2 was prepared in the same manner as described for example 1 starting with Boc-Orn(Z)-OH (3.6 g, 10 mmol) to yield 0.123 g (33%) of an oil. FAB MS: MH^+=188.0

Example 3

N-(5S-amino-6,7-dihydroxyheptyl)ethanimidamide, dihydrochloride

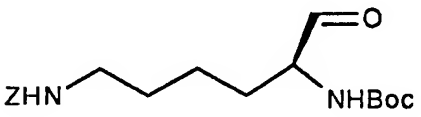


The absolute stereochemistry of the hydroxyl group at position C-6 has not been determined. The diastereomers have been separated as described below A difference in the biological activity between the diastereomers is seen.

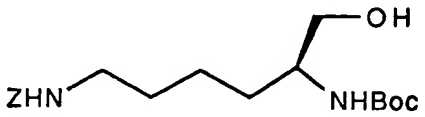
3

N-α-Boc-N-ε-Z-L-Lys-OMe (3a)

3a. To a stirring solution of cesium carbonate (32.6 g, 0.10 mol) in 150 mL DMF was added N-α-Boc-N-ε-Z-Lys (68.3 g, 0.18 mol). After 10 min, iodomethane (51.1g, 0.36 mol) was added. After 18 h, solvent was removed *in vacuo*. The resultant gum was washed with hexane and the hexane was decanted. The product was dissolved in 100 mL of DCM and filtered through a 100 x 70 mm pad of EM silica gel. The silica was washed with 900 mL DCM and 300 mL EtOAc which were combined. The solvent was removed *in vacuo* to yield 66.4 g (94 %) of product.

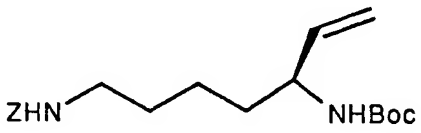


3b



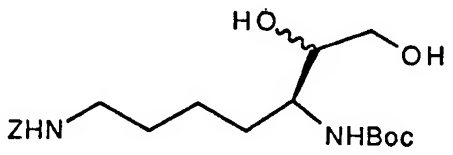
3c

3b, c. To a stirring solution of **3a** (7.9 g, 20 mmol) in 100 mL dry toluene cooled to -70°C was added dropwise over 10 min 1M DIBAL-H in toluene (40 mL, 40 mmol). After stirring an additional 20 min, the reaction was quenched with 4 mL MeOH. Upon removal of the ice bath, 150 mL of saturated solution of Rochelle salt was added to the reaction. After stirring for 1 h, the layers were separated. The aqueous layer was extracted with 2x 150 mL EtOAc. The combined organic layers were washed with 2x 200 mL H_2O, dried, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography according to Still et al. to yield 5.37g (74 %) of **3b** and 0.70 g (10 %) of **3c**. Both **3b** and **3c** were white solids.



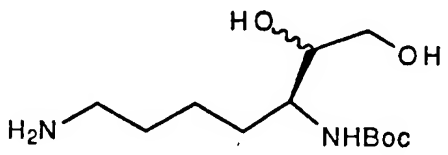
3d

3d. To a stirring suspension of methyltriphenylphosphonium bromide (2.18 g, 6.1 mmol) in 50 mL of Et_2O was added dropwise 0.5 M potassium hexamethyldisilazide in toluene (12.2 mL, 6.1 mmol). After stirring for 1.5 h, **3b** (2.22 g, 6.1 mmol) in 50 mL of Et_2O was added. After 16 h, a white solid was filtered from the reaction. The filtrate was concentrated *in vacuo*. The residue was purified by flash chromatography to yield 1.11 g (50%) of **3d**, a clear colorless gum. Anal calcd for $C_{20}H_{30}N_2O_4 \cdot 0.2\ H_2O$: C, 65.62; H, 8.37; N, 7.65. Found: C, 65.65; H, 8.07; N, 7.59.



3e

3e. To a stirring solution of **3d** (1.20 g, 3.3 mmol) in 80 mL of acetone:H_2O (3:1) was added 4-methylmorpholine N-oxide (0.64 g, 4.8 mmol) and 2.5 % OsO_4 in t-BuOH (3.4 mL, 3.4 mmol). After 18 h, 120 mL of H_2O, 8 g of celite, and 1.6 g $Na_2S_2O_4$ were added to the reaction. The reaction was filtered through a pad of wet celite. To the filtrate was added 200 mL of 1M $KHSO_4$. The filtrate was extracted with 3x 200 mL EtOAc. The combined organic layers were dried, filtered, and stripped. The residue was purified by flash chromatography to yield 0.93 g (71 %) of **3e**. Anal calcd for $C_{20}H_{32}N_2O_6 \cdot 0.25\ H_2O$: C, 59.91; H, 8.17; N, 6.99. Found: C, 59.75; H, 8.42; N, 6.77.



(3f)

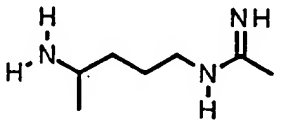
3f. Benzyloxycarbonyl protecting group was removed from **3e** (1.38 g, 3.5 mmol) by catalytic hydrogenation using Pd black as the catalyst yielding **3f** quantitatively.

3

3A, 3B. To a stirring solution of **3f** (3.90 g, 14.9 mmol) and TEA (3.3 mL, 24 mmol) in 10 mL of DMF was added methyl acetimidate (2.44 g, 22.2 mmol). After 16 h, TEA·HCl was filtered from the reaction and washed with a minimum of DMF. The filtrate was adjusted to pH 3 with 1N HCl. The filtrate was concentrated under high vacuum. The residue was applied to a reverse phase column (YMC AQ-363-10P, ODS) using a gradient of 20 % CH_3CN/0.025 % HOAc to 50 % CH_3CN/0.025 % HOAc. The two diastereomers were separated. The first eluting isomer was treated with 1N HCl for 1 h at ambient temperature. The aqueous solution was lyophilized. The yield was 0.51 g of **3A**. The second eluting isomer was treated in the same fashion to yield 0.40 g of **3B**. Anal calcd for (**3B**) $C_9H_{21}N_3O_2 \cdot 1.75HCl \cdot 0.75\ H_2O$: C, 38.52; H, 8.71; N,14.97. Found: C, 38.60; H, 8.73; N, 13.34 .

Example 4

N1-(1-iminoethyl)-1,4-pentanediamine, dihydrochloride



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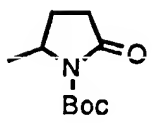
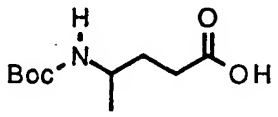


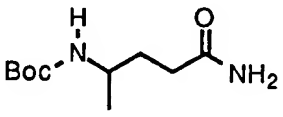
4a

4a. A solution of 5-methyl-2-pyrrolidinone (50 g, 0.5 mol), di-t-butyl dicarbonate (165 g, 0.76 mol), DMAP (62 g, 0.5 mol) and Et_3N (250 mL) in CH_2Cl_2 (250 mL) was stirred at room temperature for 24 h. The solvent was concentrated *in vacuo* and the resulting oily red solid suspended in Et_2O and filtered. The Et_2O solution was passed through a pad of silica gel. The solvent was removed to yield an orange liquid. The product was chromatographed to yield 82 g (83%) of a yellow liquid.



4 b

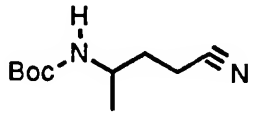
4b. Sodium hydroxide (2.24 g, 56 mmol) was added to a stirring solution of **4a** (4.0 g, 20 mmol) in THF:H_2O (175 mL:75 mL). The resulting solution was stirred for 2 h. The solvent was concentrated *in vacuo* to 75 mL. The solution was acidified with citric acid (1 M, 75 mL), extracted with EtOAc (200 mL), dried, and concentrated *in vacuo* to yield 5.36 g of an oil. The product was crystallized from Et_2O/hexane to yield 4.24 g (98%)of a white solid.



4c

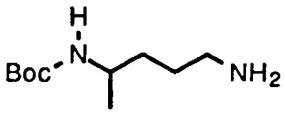
4c. To a stirred solution of **4b** (4.0 g, 18 mmol) and TEA (2.6 mL, 18 mmol) in THF (50 mL) at -10°C was added isobutyl

chloroformate (2.39 mL, 18 mmol) dropwise and the solution stirred for 20 min. Ammonium hydroxide (3.9 mL, 28%) was added and the resulting solution was stirred for 18 h allowing to warm to room temperature. The solution was concentrated *in vacuo* and the residue suspended in boiling EtOAc (80 mL) and filtered. This was repeated. The filtrate was concentrated to 30 mL and the solid collected to yield 3.82 g.



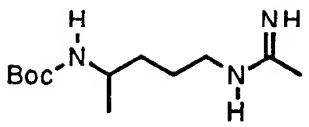
4d

4d. To a stirred solution of **4c** (3.5 g, 16 mmol) in THF (20 mL) at 0°C was added TFAA (2.5 mL, 17.5 mmol) dropwise and the solution stirred for 20 min. The solution was poured onto Et_2O (125 mL) and $NaHCO_3$ (satd, 25 mL), the layers separated and the organic solution extracted with $NaHCO_3$ (satd) and brine (satd.), dried, filtered, and concentrated *in vacuo* to yield 2.88 g of an oil. The product was vacuum distilled (bp 130°C @ 0.6 mmHg) to yield 2.2 g (69%) of a yellow liquid.



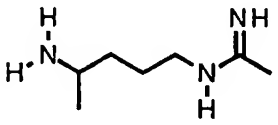
4e

4e. A solution of **4d** (1.7 g, 8.6 mmol) in EtOH was treated with H_2 (300 psi) over Raney nickel at 50°C for 8 h. The reaction mixture was filtered and concentrated *in vacuo* to yield 1.25 g (73%) of a colorless oil.



4f

4f. A solution of **4e** (1.0 g, 4.9 mmol) and ethyl acetimidate hydrochloride (0.62 g, 5 mmol) in anhyd. EtOH (25 mL) was stirred for 18 h. The reaction solution was concentrated *in vacuo* to yield 1.46 g of a white foam. This material was used in the next step without further purification.

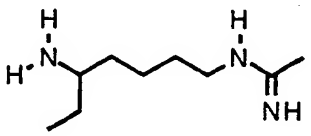


4

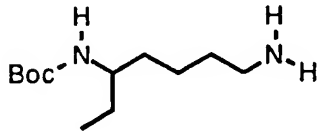
4. A solution of **4f** (1.46 g, 4.9 mmol) in acetone (25 mL) was treated with HCl (10 mL, 2 M in MeOH), and stirred for 10 min. The reaction mixture was concentrated *in vacuo* and triturated with ethanol and THF to yield an oil. Crystallization of the oil was attempted from i-propanol. The solution was concentrated *in vacuo* to obtain a foam 0.42 g (39%) which was dried. Anal. Calcd for $C_7H_{17}N_3 \cdot 2\ HCl \cdot 0.15\ H_2O \cdot 0.15$ i-PrOH: C, 39.27; H, 9.07; N, 18.44; Cl, 31.12. Found: C, 39.25; H, 9.53; N, 18.04; Cl, 31.52.

Example 5

N1-(1-iminoethyl)-1,5-heptanediamine

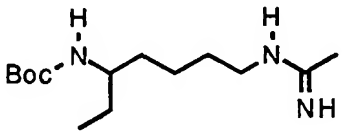


5



5a

5a. A solution of **3d** (500 mg, 1.38 mmol) in AcOH/EtOH was treated with H_2 (5 psi) over Pd black for 21 h. The reaction mixture was filtered and concentrated *in vacuo* . The residue was dissolved in CH_2Cl_2(125 mL) and extracted with NaOH (1 M), dried over Na_2SO_4, filtered, and concentrated *in vacuo* to yield 0.32 g of a white gum.



5b

5b. A solution of **5a** (0.29 g, 1.26 mmol) and ethyl acetimidate hydrochloride (0.156 g, 1.3 mmol) in EtOH (15 mL) was stirred for 18 h. The reaction solution was concentrated *in vacuo* to yield 0.40 g of a white gum. This material was used in the next step without further purification.

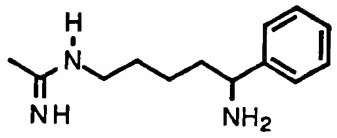
5

5. To a stirred solution of **5b** (0.40 g, 1.26 mmol) in AcOH (glacial, 10 mL) was added HCl (6.95 M in dioxane, 14 mmol). The resulting solution was stirred for 2 h. The solution was concentrated *in vacuo* to yield 0.41 g of a gum. This material was purified by reversed phase HPLC on a C-18

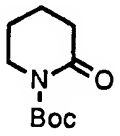
support (7:3 $CH_3CN:H_2O$) to yield 95 mg of clean product as a glass. HRMS calcd for $C_9H_{22}N_3$: 172.1814. Found: 172.1809.

Example 6

N1-(1-iminoethyl)-5-phenyl-1,5-pentanediamine

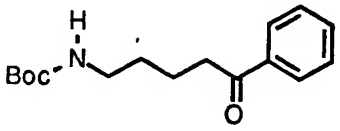


6



6a

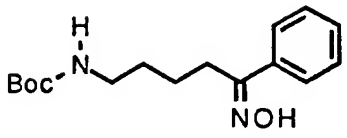
6a. The reaction for example **4a** was repeated on a 0.4 mol scale with valerolactam. The yield of the reaction was quantitative.



6b

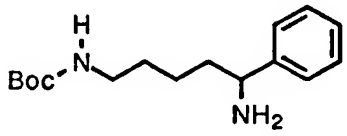
6b. A solution of **6a** (5 g, 25 mmol) in THF (125 mL) at -78°C was treated with phenylmagnesium bromide (9.5 mL, 3.0 M). The resulting solution was stirred at -72°C for 35 min then poured onto brine (satd) and extracted with Et_2O. The organic solution was dried, filtered, and concentrated *in vacuo* to yield 6.42 g of an oil. The product was chromatographed and recrystallized from hexane to yield 3.78

g (55%) of a white solid. Anal. Calcd for $C_{16}H_{23}NO_3$: C, 69.28; H, 8.36; N, 5.05. Found: C, 69.30; H, 8.84; N, 4.95.



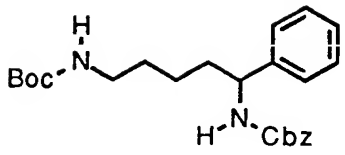
6c

6c. A stirred suspension of **6b** (0.50 g, 1.8 mmol) in EtOH (3 mL) was treated with a solution of hydroxylamine hydrochloride (0.25 g, 3.6 mmol), sodium acetate (0.25 g, 3.8 mmol) in H_2O (3 mL). The solution was refluxed for 4.5 h, during which time a solution formed. After cooling to room temperature H_2O (50 mL) was added and the mixture extracted with $CHCl_3$ (3 x 30 mL). The $CHCl_3$ extracts were combined, dried, filtered, and concentrated *in vacuo* to yield 420 mg (80%) of a white solid. Anal. Calcd for $C_{16}H_{24}N_2O_3$: C, 65.73; H, 8.27; N, 9.50. Found: C, 65.79; H, 8.79; N, 9.53.



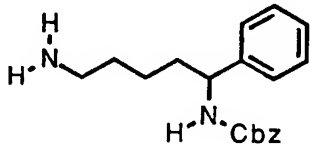
6d

6d. A solution of **6c** (3.54 g, 24 mmol) in EtOH was treated with H_2 (5 psi) over 10% Pd/C for 24 h. The reaction mixture was filtered and concentrated *in vacuo* to yield 2.61 g (77%) of a colorless oil. Anal. Calcd for $C_{16}H_{26}N_2O_2$: · 0.4 EtOH: C, 67.98; H, 9.64; N, 9.44. Found: C, 68.19; H, 9.38; N, 9.11.



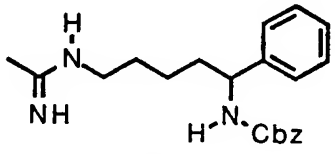
6e

6e. To a solution of **6d** (2.1 g, 7.54 mmol) in EtOAc (100 mL) in a separatory funnel was added NaOH (1 M, 60 mL) and benzylchloroformate (1.93 g, 11.31 mmol). The mixture was shaken for several minutes and the layers separated, The EtOAc solution was extracted with brine, dried, filtered, and concentrated *in vacuo* to yield an oil. The oil was chromatographed to yield 2.34 g (75%) of a white solid.



6f

6f. A solution of **6e** (2.0 g, 4.85 mmol) in CH_2Cl_2 (25 mL) at 0°C was treated with TFA (20 mL) and allowed to warm to room temperature over 1.5 h. The solution was concentrated *in vacuo* to yield a yellow oil. The oil was dissolved in $CHCl_3$ and extracted with NaOH (1 M) and brine (satd), dried over Na_2SO_4, filtered, and concentrated *in vacuo* to yield 1.35 g (89%) of a gum.



6g

6g. A solution of **6f** (1.3 g, 4.16 mmol) and ethyl acetimidate hydrochloride (0.533 g, 4.2 mmol) in EtOH (20

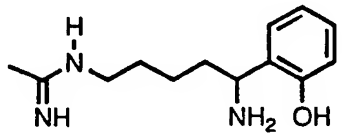
mL) was stirred for 18 h. The reaction solution was concentrated *in vacuo* to yield 1.65 g of a white foam. This material was purified by reversed phase HPLC to yield 1.09 g of a foam. Anal. Calcd for $C_{21}H_{27}N_3O_2 \cdot 1HCl \cdot 0.75\ H_2O$: C, 62.52; H, 7.37; N,10.42. Found: C, 62.82; H, 7.05; N, 10.12.

6

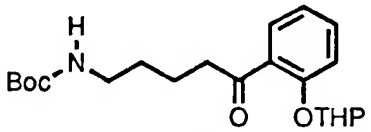
6. A solution of **6g** (0.94 g, 2.41 mmol) in AcOH was treated with H_2 (5 psi) over Pd black for 20 h. The reaction mixture was filtered and concentrated *in vacuo* . The residue was dissolved in EtOH (10 mL) and HCl/Dioxane (1 mL, 5.8 M) added and concentrated *in vacuo* to yield 0.52 g (74%) of a white powder. Anal. Calcd for $C_{13}H_{21}N_2O_2 \cdot 2\ HCl \cdot 0.75\ H_2O$. 0.2 EtOH: C, 51.10; H, 8.22; N,13.34; Cl, 22.51. Found: C, 50.98; H, 7.82; N, 13.66; Cl, 22.20.

Example 7

N-[5-amino-5-(2-hydroxyphenyl)pentyl]ethanimidamide



7



7a

7a. The reaction for example **6b** was repeated on a 25 mmol scale with 2-(tetrahydropyran-2-yloxy)phenyllithium. The 2-(tetrahydropyran-2-yloxy)phenyllithium was prepared

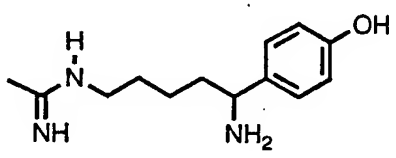
from 2-(tetrahydropyran-2-yloxy)phenyl bromide and n-BuLi in THF at -78°C. The crude product was chromatographed to yield 4.07 g (43%) of a yellow oil.

7

7. Example 7 is prepared in the same manner as described for example 6.

Example 8

N-[5-amino-5-(4-hydroxyphenyl)pentyl]ethanimidamide

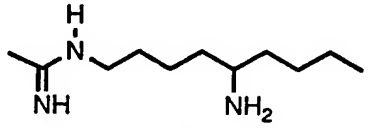


8

8. Example 8 is prepared in the same manner as described for example 6 starting with 4-(tetrahydropyran-2-yloxy)phenyllithium.

Example 9

N-(5-aminononyl)ethanimidamide

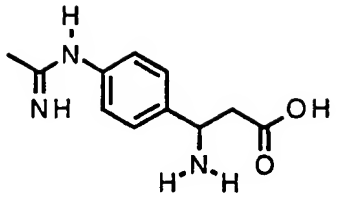


9

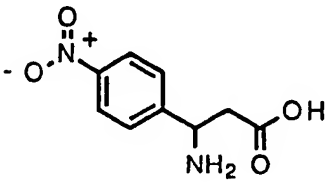
9. Example 9 is prepared in the same manner as described for example 6 starting with n-butyllithium.

Example 10

β-amino-4-[(1-iminoethyl)amino]benzenepropanoic acid, dihydrochloride hydrate

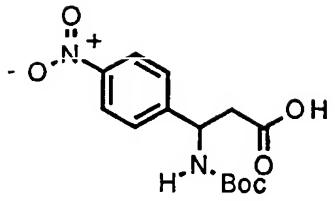


10



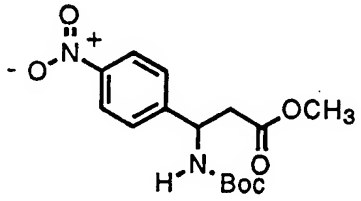
10a

10a. A mixture of 4-nitrobenzaldehyde (39 g, 0.26 mol), malonic acid (30.5 g, 0.29 mol) and ammonium acetate (49 g, 0.64 mol) in AcOH was heated at 100°C for 5 h, followed by the addition of HCl (25%, 200 mL) and continued heating at 100°C for 5 additional hours. The reaction mixture was cooled to room temperature and H_2O (300 mL) added, the resulting precipitate was filtered and washed with H_2O (100 mL). The filtrate and wash were combined and concentrated *in vacuo*, followed by the addition of H_2O (300 mL). The resulting mixture was heated on a steam bath, decolorized with carbon and filtered through celite. The pH of the solution was adjusted to 7 with NH_4OH (conc.) and the resulting precipitate collected. The solid was washed with H_2O (100 mL), methanol:H_2O (1:1, 100 mL), methanol:Et_2O (1:1, 100 mL) and Et_2O (100 mL). The solid was dried *in vacuo* to yield 29.8 g of a yellow solid.



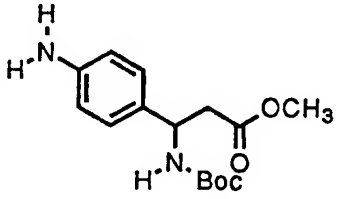
10b

10b. A solution of **10a** (5.0 g, 24 mmol), di-t-butyl dicarbonate (5.7 g, 26 mmol) in NaOH (1 M, 50 mL) and dioxane (50 mL) was stirred for 4 h. The solvent was concentrated to 50 mL to which was added EtOAc (400 mL) and $KHSO_4$ (1M, 75 mL). The layers were separated and the organic layer was washed with brine (satd.), dried, filtered, and concentrated *in vacuo* to yield 8.5 g of a yellow foam.



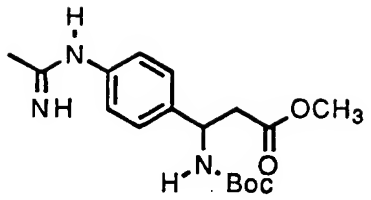
10c

10c. Example **10c** was prepared in the same manner as **3a**.



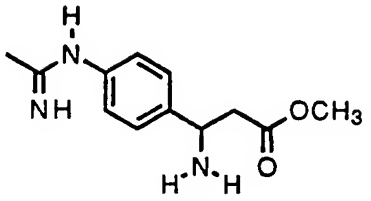
10d

10d. A solution of **10c** (8 mmol) in EtOH was treated with H_2 (5 psi) over 10% Pd/C for 18 h. The reaction mixture was filtered and concentrated *in vacuo* to yield the product.



10e

10e. The reaction for example **6g** was repeated using **10d** on a 5 mmol scale.



10f

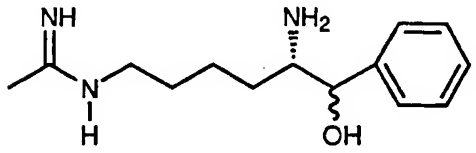
10f. A solution of **10e** (890 mg, 2.4 mmol) in CH_2Cl_2 : TFA (1:1, 50 mL) was stirred at 0°C for 15 min. The solvent was removed in vacuo and the residue was dissolved in water (100 mL) and the extracted with EA. The pH of the aqueous solution was adjusted to 11 with K_2CO_3 and extracted with CH_2Cl_2. The CH_2Cl_2 extracts were dried (Na_2SO_4) and concentrated. The residue was chromatographed on silica gel (9:1:1; ACN:H_2O:AcOH) to to yield **10f** 50 mg (5 %) as a foam. Anal. Calcd for $C_{12}H_{17}N_3O_2 \cdot 3$ AcOH·1 H_2O: C, 49.87; H, 7.21; N,9.69. Found: C, 49.61; H, 6.96; N, 9.78. HRMS calcd.: 235.1320. Found: 235.1320.

10

10. A solution of **10f** (20 mg, .5 mmol) in HCl (2N, 5 mL) was refluxed for 1 h. The solvent was removed via lyophilization to yield **10** 24 mg as a foam. Anal. Calcd for $C_{11}H_{15}N_3O_2 \cdot 2.3$ HCl·0.6 H_2O: C, 41.82; H, 5.90; N,13.30.

Example 11

α-[1-amino-5-[(1-iminoethyl)amino]pentyl]benzenemethanol hydrochloride dihydrate



11

N-α-Z-N-ε-Boc-L-Lys-N(OMe)Me (**11a**)

11a. To a stirring solution of N-α-Z-ε-Boc-L-Lys (5 g, 13.8 mmol), N,O-dimethylhydroxylamine HCl (3.9 g, 39.5 mmol),1-hydroxybenzotriazole hydrate (2 g, 14.5 mmol), and triethylamine (13.2 g, 17 mL, 130 mmol) in 75 mL of dimethylformamide (DMF) cooled in an ice bath was added EDC (2.8 g, 14.5 mmol). After stirring 55 h at ambient temperature, triethylamine hydrochloride was filtered from the reaction mixture and the filtrate was concentrated in vacuum. The residue was distributed between 150 mL of ethyl acetate (EtOAc) and 75 mL of 1M $KHSO_4$ solution. The layers were separated. The organic layer was washed with 1x 75 mL of saturated $KHCO_3$ solution and 1x 75 mL of brine and was worked up in the usual manner giving 5.3 g of **11a** (95%).

11b. To a stirring solution of **11a** (1.8 g, 4.26 mmol) and N,N,N,N-tetramethylethylendiamine (1.63 g, 2.12 mL, 14.06 mmol) in 50 mL of dry THF at -72°C was added phenyllithium, 1.8 M solution in cyclohexane, (1.18 g,7.8 mL, 14.06 mmol). After stirring at the same temperature for 2.5 h, the reaction mixture was added to 50 mL of 1M $KHSO_4$ solution and

50 mL of EtOAc. The layers were separated, the organic layer was washed with 1x 30 mL of brine and worked up in the usual manner giving 2.8 g of crude product which was purified using column chromatography. The yield of **11b** was 1.3 g (69.5%).

11c. To example **11b** (1.3 g, 2.96 mmol) dissolved in 30 mL of acetic acid was added 3 mL of 5N HCl/dioxane. The reaction was stirred for 20 min. at ambient temperature concentrated under vacuum. The residue was dried, treated with Et_2O, washed with hexane, and dried yielding 1.0 g (90.9%) of **11c**. Anal.calcd. for $C_{20}H_{24}N_2O_3 \cdot HCl \cdot 0.4\ H_2O$: C, 62.54; H, 6.77; N, 7.29; CL, 9.23. Found: C, 62.88; H, 6.80; N, 7.22; Cl, 9.18.

11d. 0.5 g of **11c** dissolved in 10 mL of water was neutralized with Na_2CO_3 (pH-9-10), the oil was extracted with 3x 15 mL of EtOAc and the organic solution was worked up in the usual manner giving 0.45 g of **11d**.

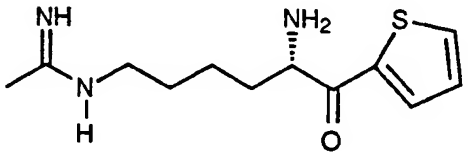
11e. A solution of **11d** (0.45 g, 1.32 mmol) and ethyl acetimidate hydrochloride (0.2 g, 1.455 mmol) in 15 mL of ethanol was adjusted to pH 9-10 using a NaOH/ethanol solution. After stirring for 1 h at ambient temperature, the reaction was acidified to pH 2 with 5N HCl/dioxane. The reaction mixture was filtered from NaCl and concentrated in vacuum. The crude product (0.5 g) was purified using reverse phase separation, giving 0.225 g of **11e** (40.91%). Anal. calcd. for $C_{22}H_{27}N_3O_3 \cdot HCl \cdot 0.5\ H_2O$: C, 61.89; H, 6.85; N, 9.84; Cl, 8.30. Found: C, 61.68; H, 6.50; N, 9.88; Cl, 8.18.

11A,11B. Example **11e** (.36 g, 0.86 mmol) was reduced under catalytic hydrogenation conditions using Pd black at 60 psi H_2 in 50% EtOH/AcOH solution for 24 h. The yield of the

crude product was 0.35 g. After reverse phase separation two products were isolated: the faster running isomer (**11A**) 0.085 g and the slower running isomer 0.1 g (**11B**). Faster running isomer analysis: calcd. for $C_{14}H_{23}N_3O$, 1.5HCl, 0.4AcOH, $2H_2O$: C, 48.82; H, 8.33; N, 11.54. Found: C, 48.56; H, 7.79; N, 11.95.

Example 12

N-[5S-amino-6-oxo-6-(2-thienyl)hexyl]ethanimidamide, hydrate



12

12a. **12a** was prepared on a 2.84 mmol scale in the same manner as described for **11b** using **11a** and 2-thiophenelithium to yield 0.6 g (47.2%) of **12a** after chromatography. Anal calcd. for: $C_{23}H_{30}N_2O_5S$: C, 61.86; H, 6.77; N, 6.27. Found: C, 61.53; H, 6.91; N, 6.12.

12b. **12b** was prepared from **12a** (0.6 g, 1.34 mmol) in the same manner as for **11c** yielding 0.4 g (85.1%).

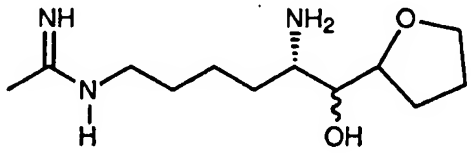
12c. **12c** was prepared from **12b** (0.4 g, 1.16 mmol) in the same manner as for **11e** to yield 0.44 g of crude product.

12. To a solution of **12c** (0.44 g, 1.14 mmol) and thioanisole (0.51 g, 0.44 mL, 2.28 mmol) in 10 mL of TFA at 0°C trimethylsilyl trifluoromethanesulfonate (TMSOTf) (0.28 g, 0.27 mL, 2.28 mmol) is added. After mixing at same

temperature for 1 h, Et_2O is added. The crude **12** is filtered and is washed with Et_2O.

Example 13

N-[5S-amino-6-hydroxy-6-(tetrahydrofuran-2-yl)hexyl]ethanimidamide

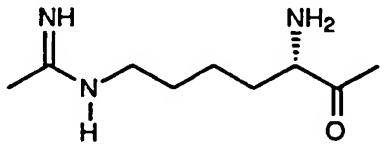


13

13. Example **13** was prepared in the same manner as example **11** on a 9.2 mmol scale starting with 2-bromofuran.

Example 14

N-(5S-amino-6-oxoheptyl)ethanimidamide, dihydrochloride



14

14a. To a stirring solution of **11a** (1.0 g, 2.4 mmol) and N,N,N,N-tetramethylethylendiamine (0.96 g, 1.25 mL, 8.3 mmol) in 30 mL of dry THF at the -72 °C was added methyllithium, 1.4 M solution in Et_2O, (5.9 mL, 8.3 mmol). After stirring at same temperature for 3 h, the reaction mixture was added to 50 mL of 1M $KHSO_4$ solution and 50 mL of EtOAc at 0 °C. The layers were separated, the organic layer was washed with 1x 30 mL of brine and worked up in the usual manner giving 2.8 g of crude product which was purified

using column chromatography. The yield of **14a** was 0.9 g (55%).

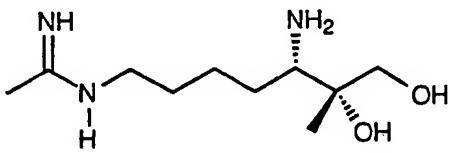
14b. To example **14a** (0.5 g, 1.2 mmol) in 10 mL of acetic acid was added 2 mL of 6N HCl/dioxane. The reaction was stirred for 20 min at ambient temperature then concentrated under vacuum. The residue was dissolved in H_2O and lyophilized yielding 0.4 g (105%) of **14b**.

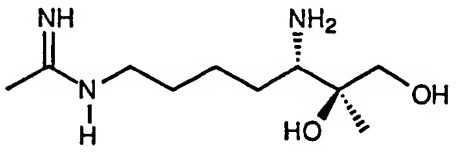
14c. To a solution of **14b** (0.4 g, 1.2 mmol) and TEA (0.56 mL, 3.9 mmol) in 10 mL of DMF was added methyl acetimidate hydrochloride (0.43 g, 3.9 mmol). After stirring for 16 h at ambient temperature, the reaction was filtered. The filtrate was concentrated under vacuum. The reaction mixture was partitioned between 15 mL 1N HCl and 20 mL DCM. The crude product from the aqueous HCl after stripping was purified using reverse phase separation, giving 0.26 g of **14c** (60.5%).

14. **14c** (0.26 g, 0.73 mmol) was reduced under catalytic hydrogenation conditions using Pd/C at 5 psi H_2 in 50% MeOH/HCl solution for 3 h. The yield of product was 0.18 g (94.7%). Analysis calcd. for $C_9H_{19}N_3O \cdot 2\ HCl \cdot H_2O$: C, 39.14; H, 8.39; N, 15.21. Found: C, 39.24; H, 8.32; N, 14.99.

Example 15

N-(5S-amino-6,7-dihydroxy-6-methylheptyl)ethanimidamide, hydrochloride dihydrate





The absolute stereochemistry of the hydroxyl group at position C-6 has not been determined. The diastereomers have been separated as described below A difference in the biological activity between the diastereomers is seen.

15

15a. To a stirring suspension of methyltriphenylphosphonium bromide (6.21 g, 17.4 mmol) in 150 mL of toluene was added dropwise 0.5 M potassium hexamethyldisilazide in toluene (35.6 mL, 17.4 mmol). After stirring for 1.5 h, **14a** (6.85 g, 17.4 mmol) in 50 mL of toluene was added to the stirring suspension cooled to -20 °C. After 5 h, the reaction was warmed to 0 °C, washed 2x 100 mL of 1M $KHSO_4$, 1x 100 mL of brine, dried, filtered, and concentrated *in vacuo*.. The residue was purified by flash chromatography to yield 5.3g (80%) of **15a**, a white solid.

15b. To a stirring solution of **15a** (3.3 g, 8.8 mmol) in 150 mL of acetone:H_2O (3:1) was added N-methylmorpholine N-oxide (2.05 g, 17.5 mmol) and 2.5 % OsO_4 in t-BuOH (9.5 mL, 0.9 mmol). After 18 h, 100 mL of H_2O, 25 g of celite, and 6 g $Na_2S_2O_4$ were added to the reaction. The reaction was filtered through a pad of wet celite. To the filtrate was added 180 mL of 1M $KHSO_4$. The filtrate was extracted with 3x 250 mL EtOAc. The combined organic layers were dried, filtered, and stripped. The residue was purified by flash chromatography to yield 3.5 g (97 %) of **15b**.

15c. To a stirring solution of **15b** (1.6 g, 3.9 mmol) in 25 mL of HOAc was added 2.5 mL of 4N HCl/dioxane. After 30 min, solvent was removed under vacuum to quantitatively recover **15c**.

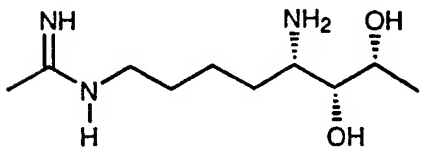
15d. Example **15d** was prepared in the same manner as described in example **14c** starting with **15c** (1.36 g, 3.9 mmol). The residue was applied to a reverse phase column (YMC AQ-363-10P, ODS) using a gradient of CH_3CN/0.025 % HOAc. The first eluting isomer, **15d-1**, weighed 0.11 g; the second eluting isomer, **15d-2** weighed 0.28 g; a mixture of the two weighed 0.18 g.

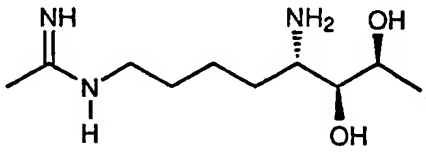
15A. Example **15A** was prepared in the same manner as described for example **14** starting with **15d-1** (1.1 g, 2.6 mmol). After lyophilization, 0.82 g of **15A** was recovered. Analysis calcd. for $C_{10}H_{23}N_3O_2 \cdot 2\ HCl \cdot 1.75\ H_2O$: C, 37.33; H, 8.93; N, 13.06. Found: C, 37.25; H, 8.70; N, 12.95.

15B. Example **15B** was prepared in the same manner as described for example **14** starting with **15d-2** (0.28 g, mmol). After lyophilization, 0.21 g of **15B** was recovered. Analysis calcd. for $C_{10}H_{23}N_3O_2 \cdot 2\ HCl \cdot 2.2\ H_2O$: C, 36.41; H, 8.98; N, 12.74. Found: C, 36.31; H, 8.97; N, 12.34.

Example 16

N-(5S-amino-6,7-dihydroxyoctyl)ethanimidamide, dihydrochloride hydrate



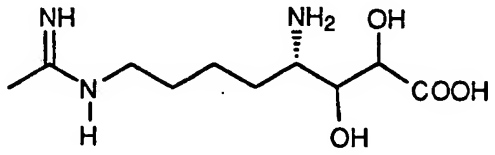


16A, 16B

16A,16B. Example **16A** and **16B** were prepared in the same manner as described for examples **3A** and **3B** starting with **3b** and ethyltriphenylphosphonium bromide. **16B**: Analysis calcd. for $C_{10}H_{23}N_3O_2 \cdot 2\ HCl \cdot 1.8\ H_2O$: C, 37.22; H, 8.93; N, 13.02. Found: C, 37.47; H, 9.05; N, 12.93.

Example 17

4S-amino-2,3-dihydroxy-8-[(1-iminoethyl)amino]octanoic acid



17a. Example **17a** is prepared starting with **3b** and (carbomethoxymethyl)triphenylphosphonium bromide.

17b. To a stirring solution of **17a** (3.3 mmol) in 80 mL of acetone:H_2O (3:1) is added N-methylmorpholine N-oxide (0.64 g, 4.8 mmol) and 2.5 % OsO_4 in t-BuOH (3.4 mL, 0.34 mmol). After 18 h, 120 mL of H_2O, 8 g of celite, and 1.6 g $Na_2S_2O_4$ are added to the reaction. The reaction is filtered through a pad of wet celite. To the filtrate is added 200 mL of 1M $KHSO_4$. The filtrate is extracted with 3x 200 mL EtOAc. The combined organic layers are dried, filtered, and stripped.

17c. Benzyloxycarbonyl protecting group is removed from **17b** by catalytic hydrogenation using Pd black as the catalyst yielding **17c** quantitatively.

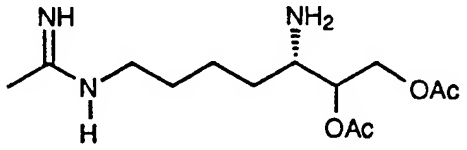
17d. To a stirring solution of **17c** (14.9 mmol) and TEA (3.3 mL, 24 mmol) in 10 mL of DMF is added methyl acetimidate (2.44 g, 22.2 mmol). After 16 h, TEA.HCl is filtered from the reaction and is washed with a minimum of DMF. The filtrate is adjusted to pH 3 with 1N HCl. The filtrate is concentrated under high vacuum. The residue is applied to a reverse phase column (YMC AQ-363-10P, ODS) using a gradient of 20 % CH_3CN/0.025 % HOAc to 50 % CH_3CN/0.025 % HOAc.

17e Example **17d** is treated with 1N HCl for 1 h at ambient temperature. The aqueous solution is lyophilized to give **17e**.

17. **17d** dissolved in 20 mL 2N HCl is refluxed for 60 min. The reaction is diluted with H_2O and lyophilized.

Example 18

N-(6,7-diacetyloxy-5S-aminoheptyl)ethanimidamide, hydrochloride monohydrate



18a To a stirring solution of **3e** (0.90 g, 2.3 mmol) and DMAP (0.61 g, 5.0 mmol) in DCM was added acetic anhydride (2.1mL, 23 mmol). After 18 h, solvent was removed under vacuum. The residue was taken up in 50 mL EtOAc which was washed with 3x 50 mL satd $KHCO_3$ solution, 1x 50 mL 1M $KHSO_4$, and 1x 50 mL H_2O. The organic layer was dried over Na_2SO_4 anhydrous, filtered, and stripped to yield 0.99 g (89%) of **18a**, a pale yellow glass. Anal calcd for $C_{24}H_{36}N_2O_8 \cdot 0.2 H_2O$: C, 59.54; H, 7.58; N, 5.79. Found: C, 59.75; H, 8.42; N, 6.77.

18b Example **18b** (0.90 g, 1.9 mmol) was prepared in same manner as **3f** to yield 0.64 g (1.8 mmol) of **18b** .

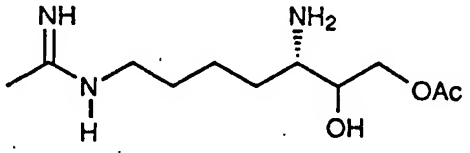
18c. To a stirring solution of **18b** (0.64 g, 1.8 mmol) in 10 mL of DMF was added a 2 mL solution of methyl acetimidate (0.10 g, 0.9 mmol) which had been neutralized with TEA (0.12 mL, 0.9 mmol) and filtered through glass wool to remove

TEA·HCl. This was repeated 4x over two hours. After stirring an additional 2 h, the reaction was adjusted to pH 3 with 1N HCl. After solvent was removed under vacuum, the crude product was purified by reverse phase chromatography. Not only was desired product **18c** (0.38 g, 51%) obtained but also the monoacetoxy compound, **19a** (0.11 g).

18. Example **18** was prepared from **18c** (0.38 g, 0.9 mmol) dissolved in 2 mL HOAc to which was added 1 mL 4N HCl/dioxane. The solvent was removed under vacuum. The residue was dissolved in H_2O and lyophilized to give **18** (0.26 g, 81%). Analysis calcd. for $C_{13}H_{25}N_3O_4 \cdot 1.75$ HCl·1 H_2O: C,42.29; H, 7.85; N, 11.38. Found: C, 42.41; H, 7.57; N, 10.68.

Example 19

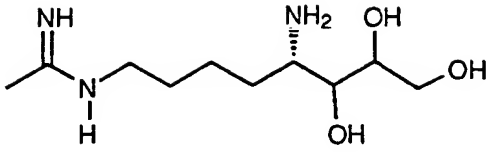
N-(5S-amino-6-hydroxy-7-acetoxyheptyl)ethanimidamide, hydrochloride monohydrate



19. Example **19a** (0.11 g, 0.29 mmol) was dissolved in 1 mL of HOAc. (see example **18c** for isolation of **19a**) To the above solution was added 1 mL of 4N HCl/dioxane. After 5 min, the solvent was removed under vacuum and the residue taken up in H_2O and lyophilized. Analysis calcd. for $C_{11}H_{23}N_3O_3 \cdot 2$ HCl·1.2 H_2O: C,38.87; H, 8.13; N, 12.36. Found: C, 38.81; H, 8.01; N, 12.07.

Example 20

N-(5S-amino-6,7,8-trihydroxyoctyl)ethanimidamide



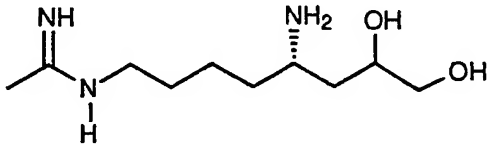
20a. **17b** dissolved in THF is treated with $LiBH_4$ to remove the benzoxycarbonyl group and reduce the ester to the alcohol.

20b To a stirring solution of **20a** (14.9 mmol) and TEA (3.3 mL, 24 mmol) in 10 mL of DMF is added methyl acetimidate (2.44 g, 22.2 mmol). After 16 h, TEA·HCl is filtered from the reaction and is washed with a minimum of DMF. The filtrate is adjusted to pH 3 with 1N HCl. The filtrate is concentrated under high vacuum. The crude product is purified by reverse phase chromatography

20 Example **20b** is treated with 1N HCl for 1 h at ambient temperature. The aqueous solution is lyophilized to give **20**.

Example 21

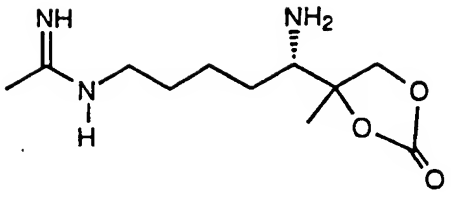
N-(5S-amino-7,8-dihydroxyoctyl)ethanimidamide



21. Example **21** is prepared in the same manner as **3A**, **3B** starting with **1b**.

Example 22

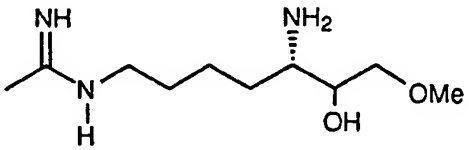
N-[5S-amino-5-(4-methyl-2-oxo-1,3-dioxolan-4-yl)pentyl]ethanimidamide



22. **15b** is treated with phosgene to generate cyclic carbonate. Example **22** is synthesized by methods described in example **15.**

Example 23

N-(5S-amino-6-hydroxy-7-methoxyheptyl)ethanimidamide



23a. To a stirring solution of **3d** (3.62 g, 10 mmol) in 25 mL of DCM was added m-chloroperbenzoic acid (2.59 g, 15 mmol). . After 16 h, solvent was removed under vacuum. The resulting residue was taken up in 100 mL of EtOAc and washed with 3x 100 mL satd $KHCO_3$ solution. The organic layer was dried, filtered, and stripped. The crude product was purified by flash column chromatography to give 2.89 g (76%) of **23a.**

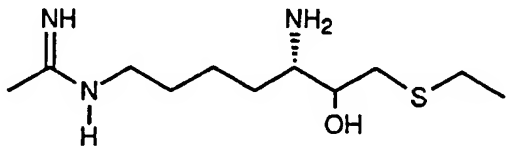
23b. Example **23b** is prepared in the manner described in *Tetrahedron Lett*, **1994**, *35*, 8977-80. To a stirring suspension of NaOMe in 15 mL of toluene-THF (2:1) cooled to -78 °C is added Et_2AlCl (3.6 mL, 3.6 mmol [1M solution]). After 30 min, 10 mL toluene solution of **23a** (0.63 g, 1.7 mmol) is added dropwise to NaOMe suspension. The reaction is quenched after 1.5 h with $Na_2SO_4 \cdot 10\ H_2O$ (5 g) and Na_2CO_3 (0.3g). After removing the ice bath, the suspension is

stirred for 1 h. The salts are filtered from reaction and the filtrate is concentrated to yield **23b**.

23. Example **23** is prepared from **23b** in the same manner as described in examples **3f** and **3**.

Example 24

N-[5S-amino-6-hydroxy-7-(ethylthio)heptyl]ethanimidamide



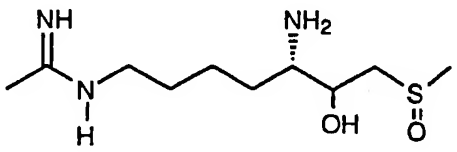
24a. To stirring ethanethiol (0.19 mL, 2.5 mmol) was added tetra-n-butylammonium fluoride. After 15 min, **23a** (0.79 g, 2.1 mmol) in 15 mL CH_3CN was added. After 16 h, the solvent was removed. The crude product is purified by flash chromatography.

24b. Example **24a** is treated with $LiAlH_4$ to remove the benzyloxycarbonyl protecting group.

24. Example **24** is prepared from **24b** using conditions described in example **3**.

Example 25

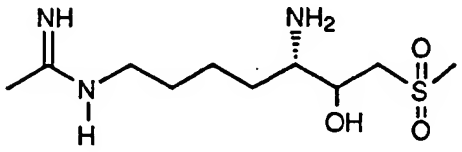
N-[5S-amino-6-hydroxy-7-(methylsulfinyl)heptyl]ethanimidamide



25. Example **25** is prepared from **24** by treatment with 30% H_2O_2 and acetic acid at room temperature for 1 h.

Example 26

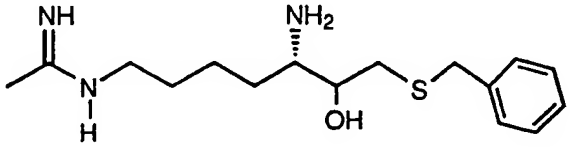
N-[5S-amino-6-hydroxy-7-(methylsulfonyl)heptyl]ethanimidamide



26. Example **26** is prepared from **24** by treatment with 30% H_2O_2 and acetic acid at 60 °C for 4 h.

Example 27

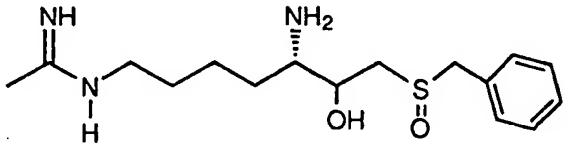
N-[5S-amino-6-hydroxy-7-[(phenylmethyl)thio]heptyl]ethanimidamide



27. Example **27** is prepared from **23a** and benzyl mercaptan in the same manner as **24.**

Example 28

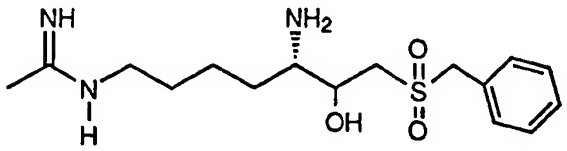
N-[5S-amino-6-hydroxy-7-[(phenylmethyl)sulfinyl]heptyl]ethanimidamide



28. Example **28** is prepared in the same manner as **25** starting with **27.**

Example 29

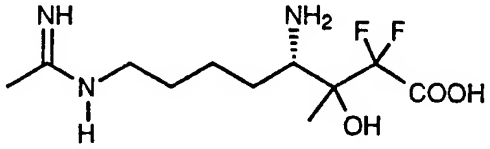
N-[5S-amino-6-hydroxy-7-[(phenylmethyl)sulfonyl]heptyl]ethanimidamide



29. Example **29** is prepared in the same manner as **26** starting with **27.**

Example 30

4S-amino-2,2-difluoro-3-hydroxy-8-[(1-iminoethyl)amino]-3-methyloctanoic acid



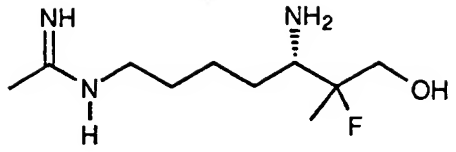
30a. To a refluxing suspension of Zn (2 mg-atm) and ethyl bromodifluoroacetate (2 mmol) in 10 mL is added dropwise a solution of **14a** (1 mmol) in 2 mL of THF. After 1 h, the reaction is cooled to room temperature. To the reaction is added 20 mL of EtOAc and 20 mL 1M $KHSO_4$. The layers are separated and the organic layer is treated in the normal manner to yield **30a.**

30b. Conditions described in example **14** are used to prepare **30b** from **30a.**

30. To remove the ethyl ester from **30b,** conditions described in example **1** are used.

Example 31

N-(5S-amino-6-fluoro-7-hydroxy-6-methylheptyl)ethanimidamide



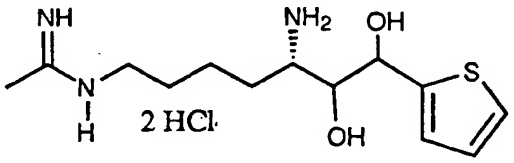
31a. To a stirring solution of **15b** (1.5 mmol) in 10 mL of pyridine is added Ph_3CCl (1.5 mmol). After 16 h, the reaction is concentrated under vacuum. The residue is taken up in 20 mL of EtOAc and is washed with 3x 20 mL 1M $KHSO_4$, 2x 20 mL saturated $KHCO_3$, and 1x 20 mL brine. The organic layer is treated in the normal manner to obtain **31a**.

31b. To a stirring solution of **31a** in dioxane is added Et_2NSF_3. After 40 h, the reaction is concentrated under vacuum and chromatographed to obtain **31b**.

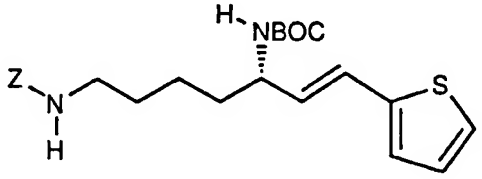
31. Using methodology described for example **15**, example **31** is synthesized from **31b**.

Example 32

N-[5S-amino-6,7-dihydroxy-7-(2-thienyl)heptyl]ethanimidamide, dihydrochloride

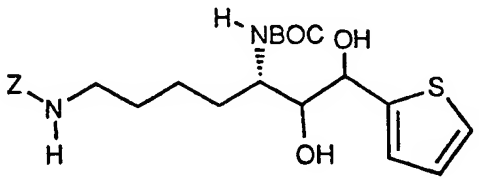


32



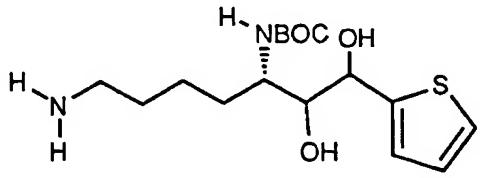
32a

32. A mixture of palladium acetate (Johnson Matthey, 0.29 mmol), tri-o-tolylphosphine (0.6 mmol), 2-bromothiophene (16.0 mmol), and triethylamine (16 mmol) is refluxed under nitrogen for 30 min. The mixture is cooled to room temperature, and **3d** (14.4 mmol) in 6 mL of acetonitrile is added. The reaction is refluxed for 24 h, cooled to room temperature, and stripped of all solvent under reduced pressure. The residue is partitioned between sat. $NaHCO_3$ and EtOAc and the organic phase is dried ($MgSO_4$), filtered, and stripped. The residue is chromatographed on silica gel to give **32a**.



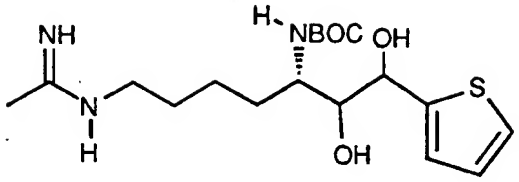
32b

32b. Osmium tetroxide is reacted with **32a** by the method used in the preparation of **3e**, to yield **32b**.



32c

32c. A solution of **32b** in AcOH is treated with H_2 (5 psi) over Pd black for 20 h. The reaction mixture is assessed by thin layer chromatography to find the extent of reaction. If necessary, fresh Pd black is added and the reaction continued. This process is repeated until the reaction is completed. The reaction mixture is filtered and concentrated *in vacuo* to yield **32c**.



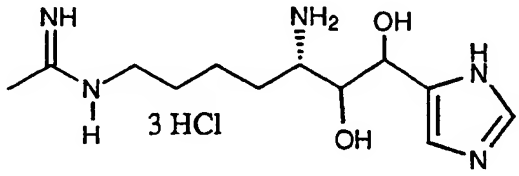
32d

32d. An equimolar solution of **32c** and ethyl acetimidate hydrochloride in EtOH is stirred for 18 h. The reaction solution is concentrated *in vacuo* to yield a white foam. This material is purified by reversed phase HPLC to yield **32d.**

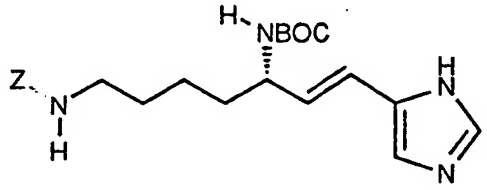
32. To a stirred solution of **32d** in AcOH (glacial) is added HCl (6.95 M in dioxane). The resulting solution is stirred for 2 h. The solution is concentrated *in vacuo* and triturated with diethyl ether to yield **32.**

Example 33

N-[5S-amino-6,7-dihydroxy-7-(1H-imidazol-5-yl)heptyl]ethanimidamide, trihydrochloride

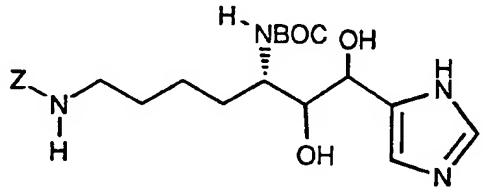


33



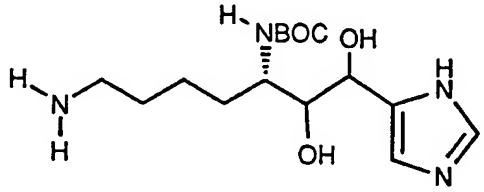
33a

33a. 4-Bromoimidazole (K&K Laboratories) is treated as described in the preparation of **32a**, replacing the 2-bromothiophene in that preparation. The product is **33a**.



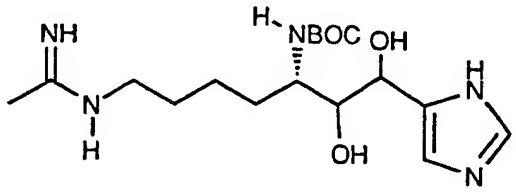
33b

33b. By the method of Example **3e**, osmium tetroxide is reacted with **33a** to yield **33b**.



33c

33c. A solution of **33b** in AcOH is treated with H_2 (5 psi) over Pd black for 20 h. The reaction mixture is filtered and concentrated *in vacuo* to yield **33c**.



33d

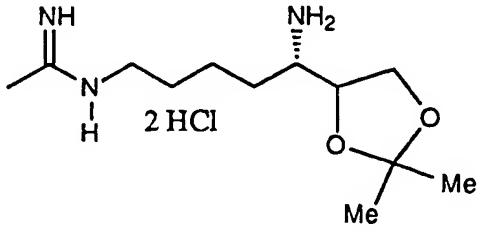
33d. An equimolar solution of **33c** and ethyl acetimidate hydrochloride in EtOH is stirred for 18 h. The reaction solution is concentrated *in vacuo* to yield a

white foam. This material is purified by reversed phase HPLC to yield **33d**.

33. To a stirred solution of **33d** in AcOH (glacial) is added HCl (6.95 M in dioxane). The resulting solution is stirred for 2 h. The solution is concentrated *in vacuo* and triturated with diethyl ether to yield **33**.

Example 34

N-[5S-amino-5-(2,2-dimethyl-1,3-dioxolan-4-yl)pentyl]ethanimidamide, dihydrochloride

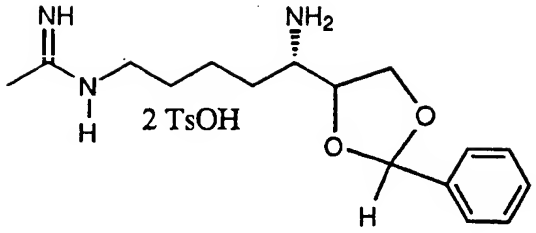


34

34. **3B** (10 mmol) is dissolved in DMF and 2,2-dimethoxypropane (20 mmol) is added, as is 6N HCl in dioxane (2 mmol). The mixture is protected from moisture and stirred overnight. It is then stripped to a residue in a rotary evaporator using an oil pump as a vacuum source. The residue is suspended in dry acetone, stirred for 30 min, and stripped again. The resulting residue is dissolved in cold water, shelled, and lyophilized to give **34**.

Example 35

N-[5S-amino-5-(2-phenyl-1,3-dioxolan-4-yl)pentyl]ethanimidamide, di(4-methylbenzenesulfonate)

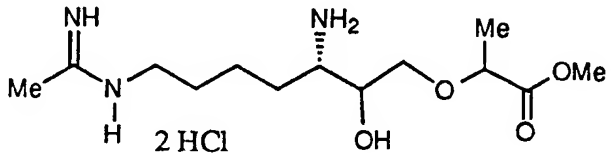


35

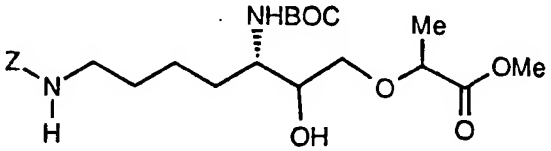
35. 3B (27.7 mmol), 1,1,1-trichloroethane (350 mL), benzaldehyde (55.4 mmol), and toluensulfonic acid monohydrate (55.4 mmol) are placed in a 500 mL round bottom single neck flask fitted with a Soxhlet extractor whose thimble is filled with 5A molecular sieves (8-12 mesh beads). The flask is immersed in an oil bath (bath temperature 120 °C) and the mixture is refluxed with vigorous stirring for 16 h. The reaction is then cooled and the mixture is stripped to a residue in a rotary evaporator using an oil pump as a vacuum source. The residue is dissolved in cold water, shelled, and lyophilized to give **35**.

Example 36

methyl 2-[[3S-amino-2-hydroxy-7-[(1-iminoethyl)amino]heptyl]oxy]propanoate, dihydrochloride

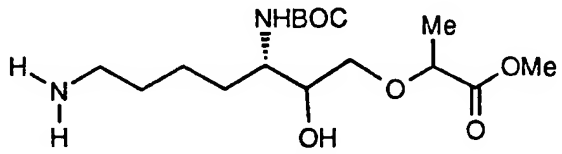


36



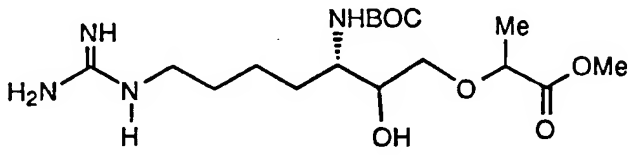
36a

36a. Sodium hydride (50% in mineral oil, 10.5 mmol) is washed twice with hexane and suspended in DMF. Methyl lactate (10 mmol) is dissolved in DMF and added carefully to the NaH suspension with stirring. The mixture is stirred for 30 min, and a solution of **23a** (9 mmol) and anhydrous zinc chloride (9 mmol) in THF is added. This mixture is immersed in a 60 °C oil bath and stirred overnight. It is then worked up to give **36a**.



36b

36b. A solution of **36a** in AcOH is treated with H_2 (5 psi) over Pd black for 20 h. The reaction mixture is filtered and concentrated *in vacuo* to yield **36b**.



36c

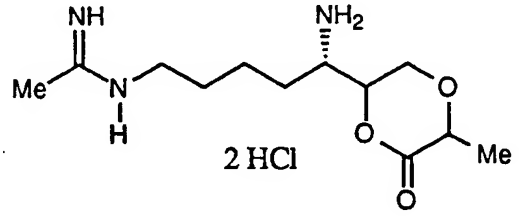
36c. An equimolar solution of **36b** and ethyl acetimidate hydrochloride in EtOH is stirred for 18 h. The reaction solution is concentrated *in vacuo* to yield a white foam. This material is purified by reversed phase HPLC to yield **36c**.

36. To a stirred solution of **36c** in AcOH (glacial) is added HCl (6.95 M in dioxane). The resulting solution is

stirred for 2 h. The solution is concentrated *in vacuo* and triturated with diethyl ether to yield **36**.

Example 37

N-[5S-amino-5-(2-methyl-3-oxo-1,4-dioxan-5-yl)pentyl]ethanimidamide, dihydrochloride

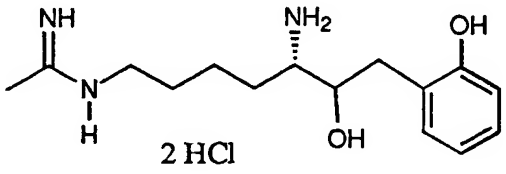


37

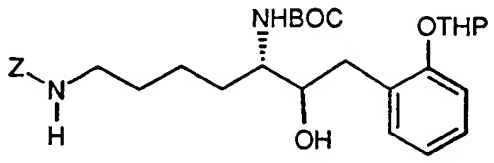
37. A solution of **36** in 1 M aqueous HCl is refluxed for two hours. The reaction is stripped to small volume, shelled, and lyophilized to give the title compound.

Example 38

N-[5S-amino-6-hydroxy-7-(2-hydroxyphenyl)heptyl]ethanimidamide, dihydrochloride



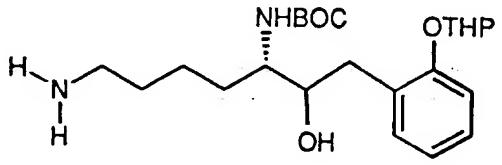
38



38a

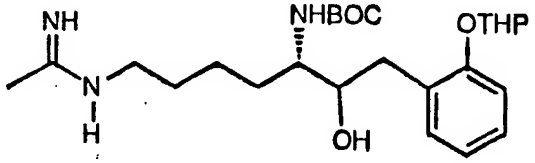
38a. 2-(Tetrahydropyran-2-yloxy)phenyllithium is prepared from 2-(tetrahydropyran-2-yloxy)phenyl bromide and

n-BuLi in THF at -78 °C. It is then reacted with **23a** in THF at -78 °C, allowing the temperature to rise to ambient temperature. The reaction mixture is worked up in the usual way to yield **38a**.



38b

38b. A solution of **38a** in AcOH is treated with H_2 (5 psi) over Pd black for 20 h. The reaction mixture is filtered and concentrated *in vacuo* to yield **38b**.



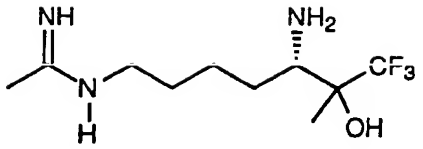
38c

38c. An equimolar solution of **38b** and ethyl acetimidate hydrochloride in EtOH is stirred for 18 h. The reaction solution is concentrated *in vacuo* to yield a white foam. This material is purified by reversed phase HPLC to yield **38c**.

38. To a stirred solution of **38c** in AcOH (glacial) is added HCl (6.95 M in dioxane). The resulting solution is stirred for 2 h. The solution is concentrated *in vacuo* and triturated with diethyl ether to yield **38**.

Example 39

N-[5S-amino-7,7,7-trifluoro-6-hydroxy-6-methylheptyl)ethanimidamide

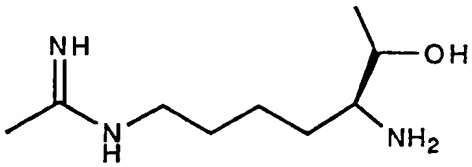


39a. To a stirring solution of CF_3I (10 mmol) in 5 mL of DMF at -40 °C is added Zn (10 mg-atm) and **14a** (0.5 mmol) in 10 mL of DMF. After stirring for 1 h at -20 °C, the reaction is warmed to room temperature and partitioned between H_2O and EtOAc. The organic layer is worked up in the usual manner to obtain desired trifluoromethyl alcohol.

39. Using conditions described in example **3**, the desired compound is obtained.

Example 40

N-(5S-amino-6-hydroxyheptyl)ethanimidamide, dihydrochloride dihydrate



40A, 40B

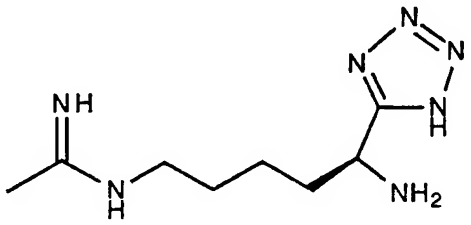
40a. To a 50 mL solution of **14a** (1.5 g, 4.0 mmol) in EtOH was added $NaBH_4$. After 2h, the reaction was concentrated under vacuum. The residue was taken up in 50 mL of EtOAc and 30 mL of H_2O. The organic was treated in the usual manner to obtain 1.5 g of **40a.**

40A,40B. Examples **40A, 40B** were prepared in same manner as described in example **15.** The first eluting fractions from the final purification by reverse phase chromatography were a single isomer (**40A**). The second eluting fractions were a mixture of two isomers (**40B**). **40A** Analysis calcd.

for $C_9H_{21}N_3O \cdot 2\ HCl \cdot 2\ H_2O$: C, 36.49; H, 9.19; N, 14.18. Found: C, 36.73; H, 8.93; N, 14.13. **40B** Analysis calcd. for $C_9H_{21}N_3O \cdot 3\ HCl \cdot 2\ H_2O$: C, 32.49; H, 8.48; N, 12.63. Found: C, 32.37; H, 8.08; N, 12.04.

Example 41

N-[5S-amino-5-(1H-tetrazol-5-yl)pentyl]ethanimidamide, dihydrochloride



41a. To a stirring solution of N-α-Boc-N-ε-Z-L-Lys. (3.8 g, 10 mmol), 2-aminopropionitrile fumarate (1.9 g, 10 mmol), 1-hydroxybenzotriazole hydrate (4.4 g, 10 mmol), and NMM (3.3 mL, 30 mmol) in 50 mL of DMF cooled in an ice bath was added (1H-1,2,3-benzotriazol-1-yloxy)-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) (4.4 g, 10mmol). After stirring 18 h at ambient temperature, the filtrate was concentrated under vacuum. The residue was distributed between 100 mL of EtOAc and 50 mL of 1M $KHSO_4$ solution. The layers were separated. The organic layer was washed with 1x 50 mL of saturated $KHCO_3$ solution and 1x 50 mL of brine and was worked up in the usual manner giving 3.9 g of **41a**.

41b. To a stirring solution of **41a** (3.9 g, 9 mmol) in 90 mL of THF was added PPh_3, DEAD, $TMSN_3$. After 24 h of stirring at ambient temperature, the reaction was cooled to 0 °C to which was added slowly 300 mL of 6% $Ce(NH_4)_2(NO_3)_6$. Additional $Ce(NH_4)_2(NO_3)_6$ was added until evolution of N_2 ceased. The layers were separated and the aqueous layer was

extracted 2x 250 mL of DCM. The combined organic layers were treated in the usual manner to yield 3.1 g of **41b**.

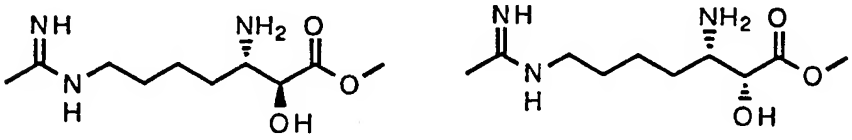
41c. To a stirring solution of **41b** (2.7 g, 5.9 mmol) in 60 mL of THF was added 7 mL of 1N NaOH. After 18 h, the reaction was concentrated under vacuum. The residue was taken up in 50 mL of EtOAC and 50 mL of 0.5N NaOH. The layers were separated and the aqueous layer was washed 2x 50 mL of EtOAc. The aqueous layer was acidified to pH 3 and extracted 3x 40 mL EtOAc. The second organic extractions were worked up in the usual manner to obtain 0.3 g of **41c**. The original organic extracts were worked up in the usual manner and they also contained product (2 g).

41. To obtain example **41**, conditions described in example **3** were used.

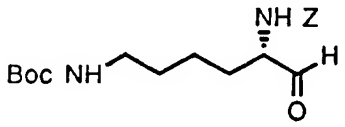
Example 42

(A) methyl 3S-amino-2S-hydroxy-7-[(1-iminoethyl)amino]heptanoate

(B) methyl 3S-amino-2R-hydroxy-7-[(1-iminoethyl)amino]heptanoate



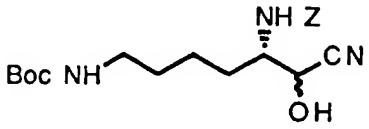
42A, 42B



42a

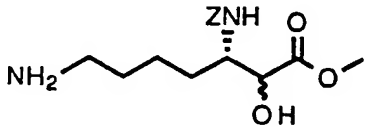
42a. To a stirring solution of N-α-Z-N-ε-Boc-L-Lys-N(OMe)Me **11a** in ether at 0°C is added $LiAlH_4$ (1.2 equiv.) in portions. The resulting solution is stirred for 1h at

0°C, then carefully quenched with $KHSO_4$ (1 M). The layers are separated and the aqueous extracted with ether. The combined organic solutions are extracted with $KHSO_4$ (1 M) and $NaHCO_3$ (satd.) dried (Na_2SO_4) and evaporated to yield the aldehyde **42a**, which is used directly in the next step.



42b

42b. A stirred mixture of the aldehyde **42a** in EtOAc and KCN (1 equiv.) in water, at 0°C, is treated with an aqueous solution of $NaHSO_3$ (satd.). The solution is stirred for 1 h, and the layers separated. The organic solution is dried over Na_2SO_4 anhydrous and concentrated to yield the resulting cyanohydrin **42b**.



42c

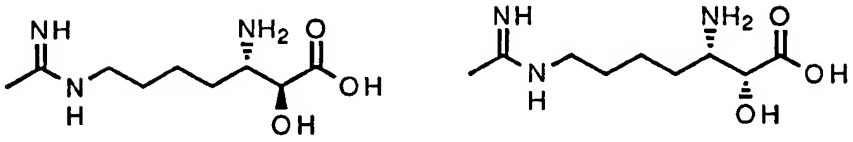
42c. The cyanohydrin **42b** is treated with methanolic/HCl to yield the methyl ester **42c**.

42A, 42B. The amine **42c** is treated with methyl acetimdate according to the procedure for **11e**. The product is then treated with Pd black according to the procedure for **11**, and the isomers separated on reversed phase HPLC to yield **42A** and **42B**.

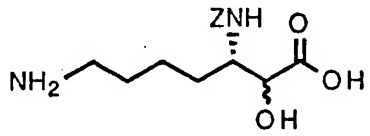
Example 43

(A) 3S-amino-2S-hydroxy-7-[(1-iminoethyl)amino]heptanoic acid

(B) 3S-amino-2R-hydroxy-7-[(1-iminoethyl)amino]heptanoic acid



43A, 43B



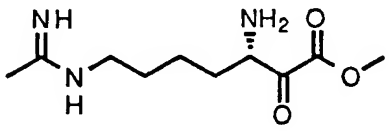
43a

43a. The cyanohydrin **42b** is treated with concentrated HCl at 0°C for 12 h. The solution is concentrated under vacuum and the ammonium chloride is removed by filtration. The residue is then dried to yield the hydroxy acid **43a.**

43A, 43B. The amine **43a** is treated with methyl acetimidate according to the procedure for **11e.** The product is then treated with Pd black according to the procedure for **11,** and the isomers separated on reversed phase HPLC to yield **43A** and **43B.**

Example 44

methyl 3S-amino-7-[(1-iminoethyl)amino]-2-oxoheptanoate



44

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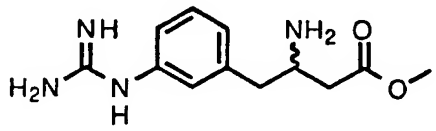
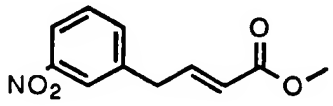
44. A solution of **42A** and **42B** in water is treated with MnO_2. The solution is filtered and concentrated to yield **44**.

Example 45

methyl 3-amino-4-[3-[(aminoiminomethyl)amino]phenyl]butanoate



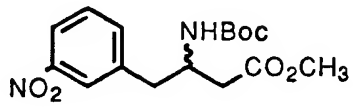
45



45a

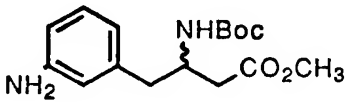
45a. To a stirred solution of oxalyl chloride (1.1 equiv.) in dry CH_2Cl_2 is slowly added at -60°C a solution of dry DMSO in CH_2Cl_2. After the solution is stirred for 5 min a solution of 3-nitrophenethyl alcohol (1 equiv.) in dry CH_2Cl_2 is added. The solution is stirred for an additional 15 min and subsequently TEA is added. After stirring for 5 min the cooling bath is removed and the solution is allowed to reach room temperature. The reaction is quenched with water. The organic layer is removed and the aqueous layer extracted with additional CH_2Cl_2. The combined organic extracts are washed with brine and water, dried(Na_2SO_4) and evaporated to yield the aldehyde. The aldehyde is treated with

carbomethoxymethyl)triphenylphosphonium bromide by the method of **17a**, to yield the ester **45a**.



45b

45b. A solution of **45a** and ammonium chloride (3 equiv.) in glacial acetic acid is refluxed for 3 h. The solvent is removed in vacuo and the residue is partitioned between EtOAc and aqueous Na_2CO_3. The layers are separated and the organic phase is dried (Na_2SO_4) and evaporated to yield the amine. The residue is taken up in THF and treated with di-t-butyl dicarbonate (1.5 equiv.) and triethylamine (1.1 equiv.). The resulting solution is refluxed for 2 h, concentrated in vacuo and purified by flash column chromatography to yield **45b**.



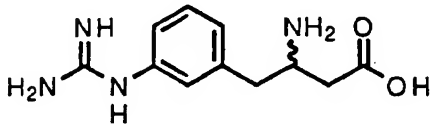
45c

45c. A solution of **45b** in methanol is hydrogenated in the presence of 10% Pd/C to yield **45c**.

45. Aminoiminomethanesulphonic acid (1.1 equiv.) is added to a solution of **45c** in methanol. The solution is stirred for 24 h. The solvent is removed and the residue is dissolved in water. The pH is adjusted to greater than 7 with NaOH. The mixture is extracted with EtOAc, dried (Na_2SO_4) and concentrated in vacuo. The residue is the treated with methanol/HCl to yield **45**.

Example 46

3-amino-4-[3-[(aminoiminomethyl)amino]phenyl]butanoic acid



46

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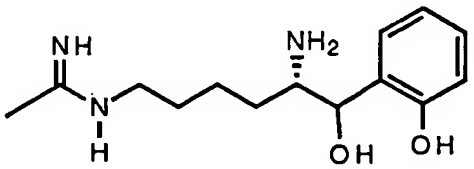
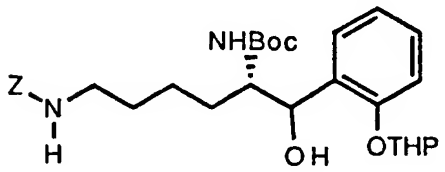
46. **45** is dissolved in 2N HCl and refluxed for 1 h. The reaction is diluted with water and lyophilized to yield **46**.

Example 47

N-[5S-amino-6-hydroxy-6-(2-hydroxyphenyl)hexyl]ethanimidamide

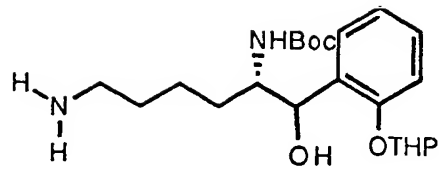


47



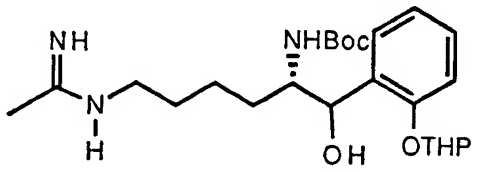
47a

47a. 2-(Tetrahydropyran-2-yloxy)phenyllithium is prepared from 2-(tetrahydropyran-2-yloxy)phenyl bromide and n-BuLi in THF at -78 °C. It is then reacted with **3b** in THF at -78 °C, allowing the temperature to rise to ambient temperature. The reaction mixture is worked up in the usual way to yield **47a**.



47b

47b. A solution of **47a** in AcOH is treated with H_2 (5 psi) over Pd black for 20 h. The reaction mixture is filtered and concentrated *in vacuo* to yield **47b.**



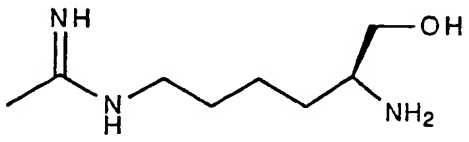
47c

47c. An equimolar solution of **47b** and ethyl acetimidate hydrochloride in EtOH is stirred for 18 h. The reaction solution is concentrated *in vacuo* to yield a white foam. This material is purified by reversed phase HPLC to yield **47c.**

47. To a stirred solution of **47c** in AcOH (glacial) is added HCl (6.95 M in dioxane). The resulting solution is stirred for 2 h. The solution is concentrated *in vacuo* and triturated with diethyl ether to yield **47.**

Example 48

N-(5S-amino-6-hydroxyhexyl)ethanimidamide

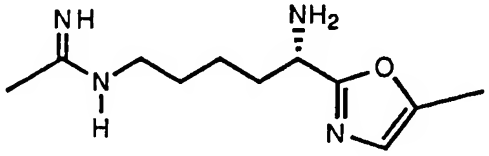


48

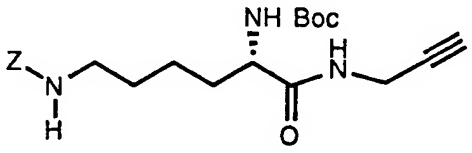
48. Example **48** was prepared using methods described in example **3** starting with **3c**. h.r.m.s. $C_8H_{19}N_3O$: 174.16.

Example 49

N-[5-amino-5-(5-methyloxazol-2-yl)pentyl]ethanimidamide, hydrochloride hydrate

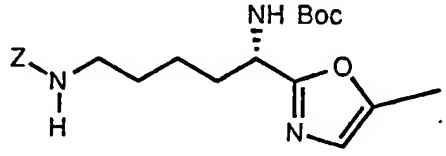


49



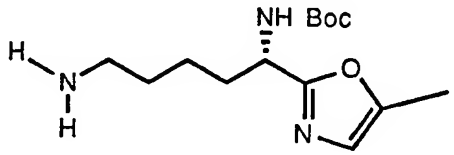
49a

To a cooled (0°C) solution of N-α-Boc-N-ε-Z-Lys (3.8 g, 10 mmol), propargylamine (550 mg, 10 mmol) and Et_3N (1 g, 10 mmol) in DMF was added HOBT (1.35 g, 10 mmol) and EDC (1.92 g, 10 mmol). The solution was allowed to gradually warm to RT over 16 h. EtOAc (500 mL) was added to the reaction solution followed by extraction with brine (4 x 100 mL), dried (Na_2SO_4) and concentrated to yield an oil. The product was crystallized from ether/hexane to yield **49a** (4.3 g) as a white solid. Anal. Calcd for $C_{22}H_{31}N_3O_2$: C, 63.29; H, 7.48; N,10.06. Found: C, 63.04; H, 7.41; N. 9.94.



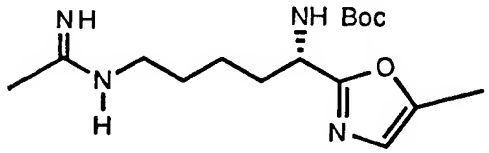
49b

A solution of **49a** (1.8 g, 4.3 mmol) and mercuric acetate (240 mg, .8 mmol) in AcOH (80 mL) was refluxed for 2 h. The solution was stirred at RT for 2h. The solvent was removed and the residue taken up in $CHCl_3$ (250 mL) and washed with NaOH (1M, 1 x 100 mL) and brine (100 mL), dried (Na_2SO_4) and evaporated to yield an oil. The product was purified by flash chromatography to yield **49b**. Anal. Calcd for $C_{22}H_{31}N_3O_2 \cdot 2\ H_2O$: C, 62.75; H, 7.52; N,9.98. Found: C, 62.40; H, 7.40; N. 9.61.



49c

A solution of **49b** (2.0 g, 4.75 mmol) in ethanol was treated with H_2 (5 psi) over Pd/C (10%) for 3 h. The reaction mixture was filtered and concentrated *in vacuo* to yield **49c**.



49d

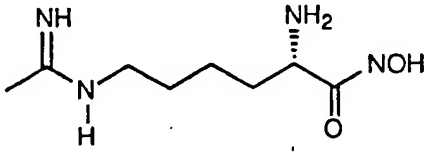
An equimolar solution of **49c** (1.0 g, 3.5 mmol)and ethyl acetimidate hydrochloride in EtOH was stirred for 18 h. The reaction solution is concentrated *in vacuo* to yield a white foam (1.2 g).

49. To a stirred solution of **49d** (1.2 g, 3.3 mmol) in AcOH (glacial, 25 mL) was added HCl (5.8 M in dioxane, 3 mL). The resulting solution was stirred for 1 h. The solution is

concentrated *in vacuo* and purified by reversed phase HPLC to yield **49**. Anal. Calcd for $C_{11}H_{20}N_4O \cdot 2.1\ HCl \cdot 1.6\ H_2O$: C: 40.07; H: 7.74; N:16.99; Cl:22.58. Found: C: 40.33; H: 8.00; N: 16.68, Cl: 22.75.

Example 50

2S-amino-N-hydroxy-6-[(1-iminoethyl)amino]hexanamide, hydrochloride



50a. Starting with N-α-Z-N-ε-Boc-Lys and O-benzylhydroxylamine hydrochloride example **50** was synthesized using conditions described in examples **11a, 11c-e, 11.** Anal. Calcd for $C_8H_{18}N_4O_2 \cdot 1.5\ HCl \cdot 0.25\ HOAc \cdot H_2O$: C: 35.21; H: 7.82; N:19.32. Found: C: 35.32; H: 7.81; N: 19.75.

Biological Data

The activity of the above listed compounds as NO synthase inhibitors has been determined in the following assays:

Citrulline Assay for Nitric Oxide Synthase

Nitric oxide synthase activity was measured by monitoring the conversion of L-[2,3-3H]-arginine to L-[2,3-3H]-citrulline (1,2). Human inducible NOS (hiNOS), human endothelial constitutive NOS (hecNOS) and human neuronal constitutive NOS (hncNOS) were each cloned from RNA extracted from human tissue. The recombinant enzymes were

expressed in insect cells using a baculovirus vector. Enzyme activity was isolated from cell extracts and partially purified by DEAE-Sepharose chromatography (2). Mouse inducible NOS (miNOS) was prepared from an extract of LPS-treated mouse RAW 264.7 cells and rat brain constitutive NOS (rcNOS) was prepared from an extract of rat cerebellum. Both preparations were partially purified by DEAE-Sepharose chromatography (2). Enzyme and inhibitors were added to give a volume of 50 µL in 50 mM Tris (pH 7.6) and the reaction initiated by the addition of 50 µL of a solution containing 50mM Tris (pH 7.6), 2.0 mg/mL bovine serum albumin, 2.0 mM DTT, 4.0 mM $CaCl_2$, 20 µM FAD, 100 µM tetrahydrobiopterin, 0.4- 2.0 mM NADPH and 60 µM L-arginine containing 0.9 µCi of L-[2,3-3H]-arginine. For constitutive NOS, calmodulin was included at a final concentration of 40-100 nM. Following incubation at 37°C for 15 minutes, the reaction was terminated by addition of 300 µL cold buffer containing 10 mM EGTA, 100 mM HEPES (pH5.5) and 1.0 mM L-citrulline. The [3H]-citrulline was separated by chromatography on Dowex 50W X-8 cation exchange resin and radioactivity quantified with a liquid scintillation counter.

1. Bredt, D. S. and Snyder, S. H. **(1990)** *Proc. Natl. Acad. Sci. U.S.A. 87*, 682-685.

2. Misko, T. P., Moore, W. M., Kasten, T. P., Nickols, G. A., Corbett, J. A., Tilton, R. G., McDaniel, M. L., Williamson, J. R. and Currie, M. G. **(1993)** *Eur. J. Pharm. 233*, 119-125.

Raw Cell Nitrite Assay

RAW 264.7 cells are plated to confluency on a 96-well tissue culture plate grown overnight (17h) in the presence of LPS to induce NOS. A row of 3-6 wells are left untreated

and served as controls for subtraction of nonspecific background. The media is removed from each well and the cells are washed twice with Krebs-Ringers-Hepes (25mM, pH 7.4) with 2 mg/ml glucose. The cells are then placed on ice and incubated with 50 µL of buffer containing L-arginine (30 µM) +/- inhibitors for 1h. The assay is initiated by warming the plate to 37°C in a water bath for 1h. Production of nitrite by intracellular iNOS is linear with time. To terminate the cellular assay, the plate of cells is placed on ice and the nitrite-containing buffer removed and analyzed for nitrite using a previously published fluorescent determination for nitrite. All values are the average of triplicate wells and are compared to a background-subtracted induced set of cells (100% value).

The following examples were assayed with the following results.

Example number	hiNOS IC_{50} (μM)	hecNOS IC_{50} (μM)	hncNOS IC_{50} (μM)	RAW cell i-NOS IC_{50}	% inhibiton iNOS at 100 μM
1	61	1990	898	300	
2					*
1d					35
4					33
6					9
2d					5
3B	12.3	8420	150	80	
15A	9.3	2350	99.6	57.3	
15B	187	6590	441		
16B	76.8	5430	670	>100	
3A					53.4
48					40.6
40A					32.6
41					28.5
49					19.4
40B					14.5
11B					4.6
10f					7.7
10					3.4
11A					*
12					*
18					7
19					54

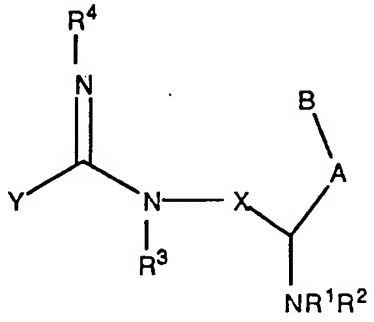
5	27
50	40.6
14	*

*At 100 μM dose, response was not seen.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

What is claimed:

1. A compound or a pharmaceutically acceptable salt, prodrug or ester therof having the formula:



Y is a hydrogen, lower alkyl radical, lower alkenyl radical,lower alkynyl radical, aromatic hydrocarbon radical, alicyclic hydrocarbon radical, amino, heterocyclyl radical in which 1 to about 4 heteroatoms are independently selected from oxygen, nitrogen and sulfur, wherein all said radicals may optionally be substituted with hydrogen, cyano, lower alkyl, nitro, amino, alicyclic hydrocarbon radicals, or aromatic hydrocarbon radicals which may be optionally substituted with lower alkyl;

X is lower alkyl radical, lower alkenyl radical, lower alkynyl radical, aromatic hydrocarbon radical, $(CH_2)_mQ(CH_2)_n$, where m= 1-3, n = 1-3, and Q is sulfur, sulfinyl, sulfonyl or oxygen, C=O, lower alkynyl radical, aromatic hydrocarbon radical, alicyclic hydrocarbon radical or heterocyclyl radicals in which 1 to about 4 heteroatoms are independently selected from oxygen, nitrogen and sulfur, wherein all said radicals are optionally substituted with hydrogen, halogen and lower alkyl;

R^1, R^2, R^3 and R^4 are independently selected from the group consisting of hydrogen and lower alkyl;

A is a lower alkyl radical, lower alkenyl radical, lower alkynyl radical, alicyclic hydrocarbon radical, C=O, aromatic hydrocarbon radical or heterocyclyl radical in which 1 to about 4 heteroatoms are independently selected from oxygen, nitrogen and sulfur, wherein all said radicals are optionally substituted with hydrogen, lower alkyl, hydroxyl, lower alkoxy, alkoxycarbonyl, alkylaryloxy, thiol, lower thioalkoxy, thioalkylaryloxy, thioaryloxy, sulfinylalkyl, sulfinylalkylaryl, sulfinylaryl, sulfonylalkyl, sulfonylalkylaryl, sulfonylaryl, halogen, aromatic hydrocarbon radicals, or alicyclic hydrocarbon radicals;

B can be hydrogen, lower alkyl radical, lower alkenyl radical, lower alkynyl radical, lower alkoxy radical, hydroxy, alkoxycarbonyl, alkylaryloxy, thiol, lower thioalkoxy, lower thioalkylaryloxy, thioaryloxy, sulfinylalkyl, sulfinylalkylaryl, sulfinylaryl, sulfonylalkyl, sulfonylalkylaryl, sulfonylaryl, aromatic hydrocarbon radical, alicyclic hydrocarbon radical, or heterocyclyl radical in which 1 to about 4 heteroatoms are independently selected from oxygen, nitrogen and sulfur wherein all said radicals are optionally substituted with hydrogen, lower alkyl, hydroxyl, lower alkoxy, halogen, aromatic hydrocarbon radicals,or alicyclic hydrocarbon radical, or

B can be $C(=O)OR^5$, $C(=O)NR^5R^6$, $P(=O)(OR^5)(OR^6)$, NHOH, $N(OH)C(=O)NR^5R^6$, $NR^5C(=O)NR^6R^7$, $NR^5C(=O)N(OH)R^6$, $C(=O)NHOH$,

where

R^5 is hydrogen, lower alkyl radical, aromatic hydrocarbon radical, or alicyclic hydrocarbon radical wherein all said

radicals are optional substituted with lower alkyl, lower alkenyl;

R^6 is hydrogen, lower alkyl radical, aromatic hydrocarbon radical, or alicyclic hydrocarbon radical wherein all said radicals are optional substituted with lower alkyl, lower alkenyl; and

R^7 is hydrogen, lower alkyl radical, aromatic hydrocarbon radical, or alicyclic hydrocarbon radical wherein all said radicals are optional substituted with lower alkyl, lower alkenyl;

with the proviso that when A is C=O, B may not be hydroxy or alkoxy.

2. The compound as recited in claim 1 wherein;

Y is a hydrogen, lower alkyl radical, lower alkenyl radical,lower alkynyl radical, aromatic hydrocarbon radical, alicyclic hydrocarbon radical, amino, heterocyclyl radical in which 1 to about 4 heteroatoms are independently selected from oxygen, nitrogen and sulfur, wherein all said radicals may optionally be substituted with hydrogen, cyano, lower alkyl, nitro, amino, alicyclic hydrocarbon radicals, or aromatic hydrocarbon radicals which may be optionally substituted with lower alkyl;

X is lower alkyl radical, lower alkenyl radical, lower alkynyl radical, aromatic hydrocarbon radical, $(CH_2)_mQ(CH_2)_n$, where m= 1-3, n = 1-3, and Q is sulfur, sulfinyl, sulfonyl or oxygen, C=O, lower alkynyl radical, aromatic hydrocarbon radical, alicyclic hydrocarbon radical or heterocyclyl radicals in which 1 to about 4 heteroatoms are independently selected from oxygen, nitrogen and sulfur,

wherein all said radicals are optionally substituted with hydrogen, halogen and lower alkyl;

R^1, R^2, R^3 and R^4 are independently selected from the group consisting of hydrogen and lower alkyl;

A is a lower alkyl radical, lower alkenyl radical,lower alkynyl radical, alicyclic hydrocarbon radical, aromatic hydrocarbon radical or heterocyclyl radical in which 1 to about 4 heteroatoms are independently selected from oxygen, nitrogen and sulfur, wherein all said radicals are optionally substituted with hydrogen, lower alkyl, hydroxyl, lower alkoxy, alkoxycarbonyl, alkylaryloxy, thiol, lower thioalkoxy, thioalkylaryloxy, thioaryloxy, sulfinylalkyl, sulfinylalkylaryl, sulfinylaryl, sulfonylalkyl, sulfonylalkylaryl, sulfonylaryl, halogen, aromatic hydrocarbon radicals, or alicyclic hydrocarbon radicals;

B can be hydrogen, lower alkoxy radical, hydroxy, alkoxycarbonyl, alkylaryloxy, thiol, lower thioalkoxy, lower thioalkylaryloxy, thioaryloxy, sulfinylalkyl, sulfinylalkylaryl, sulfinylaryl, sulfonylalkyl, sulfonylalkylaryl, sulfonylaryl,

or

B can be $C(=O)OR^5$, $C(=O)NR^5R^6$, $P(=O)(OR^5)(OR^6)$, NHOH, $N(OH)C(=O)NR^5R^6$, $NR^5C(=O)NR^6R^7$, $NR^5C(=O)N(OH)R^6$, $C(=O)NHOH$,

where

R^5 is hydrogen, lower alkyl radical, aromatic hydrocarbon radical, or alicyclic hydrocarbon radical wherein all said radicals are optional substituted with lower alkyl, lower alkenyl;

R^6 is hydrogen, lower alkyl radical, aromatic hydrocarbon radical, or alicyclic hydrocarbon radical wherein all said radicals are optional substituted with lower alkyl, lower alkenyl; and

R^7 is hydrogen, lower alkyl radical, aromatic hydrocarbon radical, or alicyclic hydrocarbon radical wherein all said radicals are optional substituted with lower alkyl, lower alkenyl.

3. The compound as recited in claim 1 wherein;

Y is hydrogen or lower alkylene;

X is lower alkylene from 3-5 carbons;

A is lower alkylene from 2-4 carbons optionally sustituted with hydroxyl

B is hydroxyl.

4. The compound as recited in claim 1 wherein;

Y is methyl;

X is butylene;

R^1, R^2, R^3, and R^4 are hydrogen;

A is ethylene or isopropylene optionally substituted with hydroxyl and

B is hydroxyl.

5. The compound as recited in claim 1 wherein;

A is lower alkyl substituted with hydrogen, lower alkyl, hydroxyl, lower alkoxy, halogen, aromatic hydrocarbon radicals, or alicyclic hydrocarbon radicals.

6. The compound as recited in claim 1 wherein the compound is selected from the group consisting of; 3S-amino-6-[(1-iminoethyl)amino]hexanoic acid; N1-(1-iminoethyl)-1,4-pentanediamine; N1-(1-iminoethyl)-1,5-heptanediamine; N1-(1-iminoethyl)-5-phenyl-1,5-pentanediamine; N-[5-amino-5-(2-hydroxyphenyl)pentyl]ethanimidamide; N-[5-amino-5-(4-hydroxyphenyl)pentyl]ethanimidamide; N-(5-aminonononyl)ethanimidamide; β-amino-4-[(1-iminoethyl)amino]benzenepropanoic acid, dihydrochloride hydrate; N-[5S-amino-6-hydroxy-6-(tetrahydrofuran-2-yl)hexyl]ethanimidamide; N-(5S-amino-6-oxoheptyl) ethanimidamide, dihydrochloride; N-(5S-amino-6,7-dihydroxy-6-methylheptyl)ethanimidamide, hydrochloride dihydrate; N-(5S-amino-6,7-dihydroxyoctyl)ethanimidamide, dihydrochloride hydrate; 4S-amino-2,3-dihydroxy-8-[(1-iminoethyl)amino]octanoic acid; N-(6,7-diacetyloxy-5S-aminoheptyl)ethanimidamide, hydrochloride monohydrate; N-(5S-amino-6-hydroxy-7-acetoxyheptyl)ethanimidamide, hydrochloride monohydrate; N-(5S-amino-6,7,8-trihydroxyoctyl)ethanimidamide; N-(5S-amino-7,8-dihydroxyoctyl)ethanimidamide; N-[5S-amino-5-(4-methyl-2-oxo-1,3-dioxolan-4-yl)pentyl]ethanimidamide; N-(5S-amino-6-hydroxy-7-methoxyheptyl)ethanimidamide; N-[5S-amino-6-hydroxy-7-(ethylthio)heptyl]ethanimidamide; N-[5S-amino-6-hydroxy-7-(methylsulfinyl)heptyl]ethanimidamide; N-[5S-amino-6-hydroxy-7-(methylsulfonyl)heptyl]ethanimidamide; N-[5S-amino-6-hydroxy-7-(phenylmethyl)thio]heptyl] ethanimidamide; N-[5S-amino-6-hydroxy-7-[(phenylmethyl) sulfinyl]heptyl]ethanimidamide; N-[5S-amino-6-hydroxy-7-[(phenylmethyl)sulfonyl]heptyl]ethanimidamide; 4S-amino-2,2-difluoro-3-hydroxy-8-[(1-iminoethyl)amino]-3-methyloctanoic acid; N-(5S-amino-6-fluoro-7-hydroxy-6-methylheptyl)ethanimidamide; -[5S-amino-6,7-dihydroxy-7-(2-

thienyl)heptyl]ethanimidamide, dihydrochloride; N-[5S-amino-6,7-dihydroxy-7-(1H-imidazol-5-yl)heptyl] ethanimidamide, trihydrochloride; N-[5S-amino-5-(2,2-dimethyl-1,3-dioxolan-4-yl)pentyl]ethanimidamide, dihydrochloride; N-[5S-amino-5-(2-phenyl-1,3-dioxolan-4-yl)pentyl]ethanimidamide, di(4-methylbenzenesulfonate); methyl 2-[[3S-amino-2-hydroxy-7-[(1-iminoethyl)amino] heptyl]oxy]propanoate, dihydrochloride; N-[5S-amino-5-(2-methyl-3-oxo-1,4-dioxan-5-yl)pentyl] ethanimidamide, dihydrochloride; N-[5S-amino-6-hydroxy-7-(2-hydroxyphenyl)heptyl]ethanimidamide, dihydrochloride; N-[5S-amino-7,7,7-trifluoro-6-hydroxy-6-methylheptyl) ethanimidamide; N-(5S-amino-6-hydroxyheptyl) ethanimidamide, dihydrochloride dihydrate; N-[5S-amino-5-(1H-tetrazol-5-yl)pentyl]ethanimidamide, dihydrochloride; (A) methyl 3S-amino-2S-hydroxy-7-[(1-iminoethyl)amino] heptanoate; (B) methyl 3S-amino-2R-hydroxy-7-[(1-iminoethyl)amino]heptanoate;(A) 3S-amino-2S-hydroxy-7-[(1-iminoethyl)amino]heptanoic acid; (B) 3S-amino-2R-hydroxy-7-[(1-iminoethyl)amino]heptanoic acid; methyl 3S-amino-7-[(1-iminoethyl)amino]-2-oxoheptanoate methyl 3-amino-4-[3-[(aminoiminomethyl)amino]phenyl]butanoate; 3-amino-4-[3-[(aminoiminomethyl)amino]phenyl]butanoic acid; N-[5S-amino-6-hydroxy-6-(2-hydroxyphenyl)hexyl]ethanimidamide; N-(5S-amino-6-hydroxyhexyl)ethanimidamide; N-[5-amino-5-(5-methyloxazol-2-yl)pentyl]ethanimidamide, hydrochloride hydrate; 2S-amino-N-hydroxy-6-[(1-iminoethyl)amino] hexanamide, hydrochloride; and a -[1-amino-5-[(1-iminoethyl)amino]pentyl]benzenemethanol hydrochloride dihydrate.

7. The compound as recited in claim 1 wherein the compound is 3S-amino-7-[(1-iminoethyl)amino]heptanoic acid; N-(5S-amino-6,7-dihydroxyheptyl)ethanimidamide,

dihydrochloride; and N-(5S-amino-6,7-dihydroxy-6-methylheptyl)ethanimidamide, hydrochloride dihydrate.

8. A pharmaceutical composition comprising a compound as recited in claim 1 together with a pharmaceutically acceptable carrier.

9. A pharmaceutical composition comprising a compound as recited in claim 2 together with a pharmaceutically acceptable carrier.

10. A pharmaceutical composition comprising a compound as recited in claim 3,4,5,6 or 7 together with a pharmaceutically acceptable carrier.

11. A method of inhibiting nitric oxide synthesis in a subject in need of such inhibition by administering a therapeutically effective amount of the compound as is recited in Claim 1.

12. A method of inhibiting nitric oxide synthesis in a subject in need of such inhibition by administering a therapeutically effective amount of the compound as is recited in Claim 2.

13. A method of inhibiting nitric oxide synthesis in a subject in need of such inhibition by administering a therapeutically effective amount of the compound as is recited in Claim 3,4,5,6 or 7.

INTERNATIONAL SEARCH REPORT

Internat Application No
PCT/US 95/02669

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07C257/14 C07C279/18 A61K31/155 A61K31/195 C07D333/22
C07D307/14 C07C317/28 C07C323/41 C07D333/20 C07D233/54
C07D317/28 C07D319/12 C07C259/06 C07D257/04 C07D263/32

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CANADIAN JOURNAL OF CHEMISTRY, vol.72, no.1, January 1994, OTTAWA CA pages 6 - 11 S. J. GOULD 'Nucleoside intermediates in plasticidin S biosynthesis identified by the in vivo use of enzyme inhibitors' see page 7, left column, example 2a ---	1,2,5
X	TETRAHEDRON, vol.49, no.1, 1993, OXFORD GB pages 13 - 28 Y. FUNABASHI ET AL. 'A new anti-MRSA dipeptide, TAN-1057 A' see page 16, compound 2 ---	1,2,5
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 June 1995	30. 06. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Seufert, G

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL AND PHARMACEUTICAL BULLETIN., vol.36, no.3, 1988, TOKYO JP pages 1205 - 1209 H. TSUNEMATSU ET AL. '.beta.-naphthylamides of guanidinophenyl amino acids' see figure 1 ---	1,2
X	CHEMICAL ABSTRACTS, vol. 107, no. 5, 3 August 1987, Columbus, Ohio, US; abstract no. 40336, W. STUEBER 'Oligopeptidylarginol derivatives and their homologs, their use as antithrombotics and agents containing them' page 758 ; see RN 109056-07-7, Guanidine, (4-amino-5-hydroxypentyl)-, (S)-, bis(trifluoroacetate) (salt & EP,A,0 192 135 ---	1,2,5
X	TETRAHEDRON LETTERS, vol.27, no.33, 1986, OXFORD GB pages 3815 - 3818 P. C. PRABHAKARAN ET AL 'Studies on nitrogen metabolism using 13C-NMR spectroskopy' see page 3816, compound 2 ---	1,2,5
X	CHEMICAL ABSTRACTS, vol. 63, no. 5, 30 August 1965, Columbus, Ohio, US; abstract no. 5641d, NOBORU OTAKE ET AL. 'Structure of blasticidin S' see abstract & TETRAHEDRON LETTERS, no.19, 1965, OXFORD GB pages 1411 - 1419 ---	1,2,5
X	CHEMICAL ABSTRACTS, vol. 97, no. 5, 2 August 1982, Columbus, Ohio, US; abstract no. 38442, E. KOLTAI ET AL. 'A novel synthesis of ethanolamine-2-14c' page 525 ; see RN 82300-01-4, Guanidine, (4-amino-5-hydroxypentyl)-, monohydrobromide & J. LABELLED COMPD. RADIOPHARM., vol.19, no.1, 1982 pages 7 - 11 --- -/--	1,2,5

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 76, no. 9, 28 February 1972, Columbus, Ohio, US; abstract no. 43768, M. KISUMI ET AL. 'production of L-arginine by arginine hydroxamate resistent mutants of Bacillus subtilis' page 190 ; see RN 35832-00-9, Pentanamide, 2-amino-5-[(aminoiminomethyl)amino]-N-hydroxy-, hydrochloride, (S)- & APPL. MICROBIOL., vol.22, no.6, 1971 pages 987 - 991	1,2
X	CHEMICAL ABSTRACTS, vol. 118, no. 8, 22 February 1993, Columbus, Ohio, US; abstract no. 72838, F. H. WALTERS, QIU HONGCHUN 'The use of a mixture design to analyze the effects of different alcohols on the paper chromatographic separation of amino acids and amino acid hydroxamates' page 875 ; see RN 25125-96-6, Pentanamide, 2-amino-5-[(aminoiminomethyl)amino]-N-hydroxy-, (S)- & ANAL. LETT., vol.26, no.1, 1983 pages 183 - 195	1,2
X	CHEMICAL ABSTRACTS, vol. 64, no. 12, 6 June 1966, Columbus, Ohio, US; abstract no. 17593h, NOBORU OTAKE ET AL. 'Chemical studies of blastidin S' see RN 5738-55-6, Valeric acid, 3-amino-5-(1-methylguanidino)-, dihydrochloride & AGR. BIOL. CHEM., vol.30, no.2, 1966 pages 132 - 141	1,2,5
X	CHEMICAL ABSTRACTS, vol. 115, no. 3, 22 July 1991, Columbus, Ohio, US; abstract no. 29868, E. LEPORATI, G. NARDI 'Properties of aminohydroxamic acid and their metal chelates.' page 832 ; see RN 5699-67-2, Pentanamide, 2-amino-5-[(aminoiminomethyl)amino]-N-hydroxy- & GAZZ. CHIM. ITAL., vol.121, no.3, 1991 pages 147 - 154	1,2

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 104, no. 23, 9 June 1986, Columbus, Ohio, US; abstract no. 202858, I. YAMAGUCHI ET AL. 'Substrate binding by blasticidin S deaminase, an aminohydrolase for novel 4-aminopyrimidine nucleocides' page 351 ; see RN 3730-67-4, Pentanoic acid, 3-amino-5-[[imino(methylamino)methyl]amino]-, (S) & PESTIC. BIOCHEM. PHYSIOL., vol.25, no.1, 1986 pages 54 - 62 ---	1,2,5
A	US,A,5 132 453 (O. W. GRIFFITH) 21 July 1992 cited in the application see abstract ---	1,8-13
A	WO,A,93 24126 (CORNELL RESEARCH FOUNDATION) 9 December 1993 see abstract ---	
A	WO,A,93 13055 (THE WELLCOME FOUNDATION) 8 July 1993 cited in the application see page 3, line 1 - page 4, line 14; examples -----	1,8-13

INTERNATIONAL SEARCH REPORT

I. .iational application No.
PCT/US 95/ 02669

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

The vast number of theoretically conceivable compounds resulting from the combination of all claimed substituents precludes a comprehensive search. For econimical reasons the search has been carried out for compounds structurally similar to the examples, especially for compounds with the ./.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/

A-B residue as described in the examples and NR1R2 = NH2.
(See Guidelines Exam. Part. B, Chapt. III 3.6, 3.7)

Claims searched incompletely: 1,2,5,8-13

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna Application No
PCT/US 95/02669

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0192135	27-08-86	DE-A-	3505555	11-09-86
		AU-B-	589159	05-10-89
		AU-A-	5372786	21-08-86
		CA-A-	1269200	15-05-90
		JP-A-	61189298	22-08-86
		US-A-	4713369	15-12-87
US-A-5132453	21-07-92	AU-A-	1683292	21-10-92
		EP-A-	0582630	16-02-94
		JP-T-	6506220	14-07-94
		NZ-A-	242029	25-03-94
		WO-A-	9216666	01-10-92
		US-A-	5273875	28-12-93
WO-A-9324126	09-12-93	US-A-	5281627	25-01-94
		EP-A-	0649303	26-04-95
WO-A-9313055	08-07-93	AU-B-	3169293	28-07-93
		EP-A-	0618898	12-10-94
		JP-T-	7502512	16-03-95